











# JOURNAL OF AGRICULTURAL RESEARCH

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WASHINGTON, D. C.



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## ERRATA AND AUTHORS' EMENDATIONS

- Page 119, line 9, should read "The optimum reaction for growth" instead of "The optimum temperature for growth."
- Page 160, line 12 from bottom, should read "rhabditiform" instead of "rhabditiform."
- Page 214, line 13, should read "Old cankers" instead of "Old cankers."
- Page 464, line 9 from bottom, should read "respectively" instead of "respective."
- Page 467, line 35, should read "than are preexisting" instead of "then preexisting."
- Page 325, line 12 from bottom, should read "an organism endemic in nature" instead of "an organism more or less endemic."

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NO. 1

## WORK AND PARASITISM OF THE MEDITERRANEAN FRUIT FLY IN HAWAII DURING 1919 AND 1920<sup>1</sup>

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Control by parasites has been the only method of combating the Mediterranean fruit fly (*Ceratitis capitata* Weidemann) in Hawaii that has met with any degree of success since its introduction in 1910. In 1911 the Territorial government inaugurated a clean-culture campaign, which was taken over by the Federal Bureau of Entomology in 1912 and continued until 1914. This campaign consisted of gathering and destroying all host fruits in Honolulu. During its investigations of the fruit fly from 1912 to 1914 the Bureau of Entomology tried extensive spraying experiments with poisoned sprays, endeavoring to kill the adult flies. Both of these methods of control failed because of the great abundance and variety of host fruits, there being over 70 varieties in Honolulu alone, some of which are bearing at all seasons of the year.

During the time these experiments were being made the Board of Agriculture and Forestry of the Territory of Hawaii engaged Prof. F. Silvestri, an Italian entomologist, to travel in Africa and Australia in search of fruit-fly parasites. In May, 1913, he arrived in Honolulu with a few living specimens of the opiine larval parasites *Opius humilis* Silvestri from Africa and *Diachasma tryoni* Cameron from Australia. In 1914 the Territorial government sent D. T. Fullaway and J. C. Bridwell to Africa to search for additional parasites. As a result of this expedition two larval parasites, an opiine (*Diachasma fullawayi* Silvestri) and a chalcid (*Tetrastichus giffardianus* Silvestri) were introduced in October of that year. All four of these parasites soon became established and were distributed to all the larger islands of the Hawaiian group.

The Bureau of Entomology, during its studies of the Mediterranean fruit fly in Hawaii and in conjunction with its quarantine work, has had an exceptional opportunity to observe the results achieved by these parasites since their introduction. A series of papers has been published,<sup>2</sup> giving yearly records of the work done by them, as individual

<sup>1</sup> Accepted for publication July 17, 1921.

<sup>2</sup> BACK, E. A., and PEMBERTON, C. E. PARASITISM AMONG THE LARVAE OF THE MEDITERRANEAN FRUIT FLY IN HAWAII DURING 1914. In *Bien. Rpt. Bd. Comrs. Agr. and Forestry Hawaii*, 1913-14, p. 153-161. 1915.

— PARASITISM AMONG THE LARVAE OF THE MEDITERRANEAN FRUIT FLY (*C. CAPITATA*) IN HAWAII DURING 1915. In *Jour. Econ. Ent.*, v. 2, no. 2, p. 369-374. 1916.

PEMBERTON, C. E., and WILLARD, H. F. FRUIT-FLY PARASITISM IN HAWAII DURING 1916. In *Jour. Agr. Research*, v. 12, no. 2, p. 103-108. 1918.

— WORK AND PARASITISM OF THE MEDITERRANEAN FRUIT FLY IN HAWAII DURING 1917. In *Jour. Agr. Research*, v. 14, no. 11, p. 595-603. 1918.

WILLARD, H. F. WORK AND PARASITISM OF THE MEDITERRANEAN FRUIT FLY IN HAWAII DURING 1918. In *Jour. Agr. Research*, v. 18, no. 8, p. 441-456. 1920. Literature cited, p. 446.

species and collectively, and the extent of infestation of different fruits by *Ceratitis capitata*. The present paper is a continuation of these records for the years 1919 and 1920.

TABLE I.—Extent of infestation of host fruits by larvae of *Ceratitis capitata* in Hawaii during 1919 and 1920

Host fruit.	Number of fruits collected.		Number of <i>C. capitata</i> larvae emerging.		Average number of larvae per fruit.	
	1919	1920	1919	1920	1919	1920
Indian almond ( <i>Terminalia catappa</i> ).....	35,716	34,066	300,394	187,811	8.4	5.5
Mango ( <i>Mangifera indica</i> ).....	1,595	1,787	5,857	5,212	3.7	3.5
Coffee ( <i>Coffea arabica</i> ).....	16,955	4,080	6,985	2,441	.4	.6
Strawberry guava ( <i>Psidium cattleianum</i> ).....	20,539	22,133	25,266	25,662	1.2	1.2
Black myrobalan ( <i>Terminalia chebula</i> ).....	8,199	3,373	38,359	23,199	4.7	6.9
Peach ( <i>Amygdalus persica</i> ).....	385	10	5,220	150	13.6	15.0
Satin-leaf ( <i>Chrysophyllum olivaceum</i> ).....	229	851	1,136	3,748	5.0	4.7
Rose-apple ( <i>Eugenia jambos</i> ).....	4,225	.....	49,329	.....	11.5	.....
French cherry ( <i>Eugenia uniflora</i> ).....	8,671	5,725	7,643	8,246	.9	1.4
West Indian medlar ( <i>Elaeagnus elengi</i> ).....	.....	2,287	.....	834	.....	.4
Karant ( <i>Calophyllum inophyllum</i> ).....	450	438	2,682	2,147	6.0	4.5
Yellow oleander ( <i>Thevetia nerifolia</i> ).....	1,479	2,307	2,462	4,590	1.7	1.5
Carambola ( <i>Averrhoa carambola</i> ).....	153	106	7	22	.05	.1
Chinese orange ( <i>Citrus</i> sp.).....	21,804	40,260	53,870	94,614	2.5	2.2
Guava ( <i>Psidium guajava</i> ).....	6,675	4,051	65,732	29,168	9.8	7.1
Loquat ( <i>Eriobotrya japonica</i> ).....	1,690	.....	5,827	.....	3.4	.....
Nerankia emarginata.....	.....	194	.....	28	.....	.....
Orange ( <i>Citrus aurantium</i> ).....	216	611	710	3,649	3.3	6.1
Waiawi ( <i>Psidium guajava</i> var. <i>peruvianum</i> ).....	.....	947	.....	581	.....	.1
Lime ( <i>Citrus medica limetta</i> ).....	.....	154	.....	182	.....	1.2
Tangerine ( <i>Citrus nobilis</i> ).....	.....	809	.....	768	.....	.9

Table I gives data which show the average infestation per fruit of 21 different varieties collected about Honolulu in 1919 and 1920 and indicates in a general way the abundance of adults of *Ceratitis capitata* for those years. A comparison of this table with Table I in the records of parasitism for 1918,<sup>2</sup> would indicate a reduction of this pest during the past three years. For the year 1919 the average infestation of 9 varieties of host fruits was less than during 1918, and greater in 5. For the year 1920 it was less in 9 varieties, the same in 1, and greater in 6 than in 1918. It is encouraging to see that many of these reductions in infestation occurred in the most preferred hosts of the fly, notably the peach (*Amygdalus persica*) and Indian almond (*Terminalia catappa*). The peach has always been the most heavily infested fruit in Hawaii, and the average infestation for 1919 was less than for any year since the introduction of parasites, and over 33 per cent less than in 1918. The Indian almond can be found in all sections of Honolulu and is much preferred as a breeding place by the fly. It bears prolifically, and its infested fruits can be

<sup>2</sup> WILLARD, H. F. OP. CIT.

secured during almost every month of the year. Consequently, Indian almond has been used more than any other fruit in securing parasitism records of the fruit fly. Average infestation records of this fruit alone, of which about 30,000 are collected yearly, are a good guide to the abundance of *C. capitata* in this locality. In 1919 and 1920 this average decreased 15 per cent and 44 per cent, respectively, over that of 1918. These are the first important decreases in infestation of preferred host fruits that have taken place since parasitism records were started.

Table II, which records the parasitism of the larvæ in each host fruit by the month, reveals interesting information relative to *Diachasma fullawayi* and *Tetrastichus giffardianus*. Prior to 1920 the former had a tendency to attack its host freely in only a few fruits, namely, strawberry guava (*Psidium cattleianum*), coffee (*Coffea arabica*), French cherry (*Eugenia uniflora*), and yellow oleander (*Thevetia nerifolia*). Other fruits occasionally yielded larvæ that were parasitized by *D. fullawayi*; but the 1920 records show larvæ, in nearly all fruits under observation, to be freely attacked, especially during the latter part of the year. It caused the death of 12.1 per cent of all larvæ during the year (Table IV), which is more than double its percentage of parasitism during any of the previous five years, with the exception of 1917, when it was 7.3. *T. giffardianus* has also shown an increase over previous years, although not so great an increase as *D. fullawayi*. It has proved its value by its ability to attack its host in fleshy fruits, where the fruit-fly maggots are protected to a considerable extent from the opiine parasites. *T. giffardianus* attacks its prey within the fruit, after entering through a crack or other opening, by attaching itself to the larva while ovipositing. In this manner it can reach many larvæ which are out of reach of the opiine parasites, which oviposit only in larvæ near the surface, by piercing the skin and pulp of the fruit with their ovipositors. If fruits with thin skin and shallow pulp, like the Indian almond and coffee, were the only ones grown in Hawaii, the opiine parasites now there would probably control the fruit fly; but the fleshy fruits, such as the guava (*P. guajava*), of which there are thousands of acres and in which these parasites work with difficulty, serve as a constant source of supply of adult fruit flies. It is interesting to note that *T. giffardianus* attacked the larvæ in guava very freely during 1920. In six out of the nine months during which records were obtained it destroyed more larvæ in this fruit than the other three parasites combined. The records for 1919 and 1920 have greatly enhanced the value of this parasite.

TABLE II.—Percentage of parasitism of larvae of *Ceratitis capitata* in Hawaii in 1919 and 1920

Host fruit.	Month	Number of larvae emerging during first 1 to 6 days.	Percentage of parasitism.										Total.	
			<i>Opius humilis</i> .		<i>Diachasma tryoni</i> .		<i>Diachasma fullawayi</i> .		<i>Tetrastichus giffordianus</i> .					
			1919	1920	1919	1920	1919	1920	1919	1920				
Indian almond	Jan.	6,132	399	9.9	12.6	9.8	19.7				1.4	9.1	21.1	41.4
	Feb.	3,077	255	15.2	14.9	12.2	4				3.2	1.7	41.5	20.0
	Mar.	197	121	39.5	23.9	13.7	2.1	2.0	0.7	4.1	4.1	30.3	28.8	
	Apr.	122	1,199	18.9	16.2	12.1	18.1			6.6	3.3	3.4	35.3	43.6
	May		6,811		28.7		31.6			4		3.1		61.8
	June	443	240	2.6	50.0	26.2	23.8			14.3	2.9	40.1	76.7	
	July	7,093	4,771	46.4	5.8	34.1	16.3			15.0	7.6	8.1	65.9	42.2
	Aug.	7,712	4,496	7.9	9.5	8.4	13.4			24.1	14.9	11.6	31.7	38.6
	Sept.	7,652	6,489	5.7	5.2	16.4	29.1	2.9	19.4	8.6	21.5	33.2	75.4	
	Oct.	8,617	3,993	4.2	2.4	13.2	36.4	4.4	6.7	6.4	6.0	26.1	75.5	
	Nov.	7,200	729	8.0	2.9	29.7	49.1	2	10.1	8.2	12.0	40.1	83.2	
	Mango	Dec.	4,193	96	11.3	1.7	41.6	11.7			20.0	8.9	13.1	65.8
Jan.		222	123	2.7	2.4	12.2	7.1			3.9	4.0	1.6	15.2	11.7
May		572	312	4.0	7	16.3	7.1						15.6	2.1
June		254	491	3.6	2.0	15.8	12.6	1.1	6.3	4.2	1.5	24.7	23.0	
July		107		1.6		9.6		1.2		6.0			17.4	
Strawberries guava	Jan.		31		12.3					13.1		15.2		54.6
	Feb.		127		19.5					9.1		2.8		61.6
	Mar.	12	441	16.6	55.5		5.2			3.1		1.6	16.6	95.4
	Apr.	153		46.0								7		48.7
	May	3,433	399	2.6	1.2	33.4	6.5	1.5	9.4	2.1	3.9	59.0		21.0
	June	7,449			7.1		31.5		1.8			3.8		61.9
	July		1,276		4		40.8		17.5		7.9			74.8
	Aug.		126		4.4		51.4		15.6		2.8			74.1
	Sept.		495		3.2		9.1		66.3		5.9			84.5
	Oct.		791	96.1	1.0		33.5	3.0	7.4	35.3			44.0	36.9
Coffee	Feb.		293		2.1		33.6		26.1					65.7
	Mar.		390		1.3		71.8							74.7
	Apr.		9		33.3			22.2						55.5
	May		19											0
	July		155		3.8		26.5						34.3	
	Aug.		62		8		24		90.9					95.1
Black myrotalan	Apr.		710		6		4						1.1	
	Aug.		153		3.3				9.1		2.6		13.0	
	Sept.		1,137		4.4		4		1.3		3.9		10.0	
	Oct.		1,425	72	16.9		4.6	4.9	8.2	5.8			30.2	14.3
	Nov.		1,249	313	3.3		2.9	5.1	39.1	9.1	5.8	21.5	35.0	
Peach	Dec.		311	346	16.1		1.0	7.0	1.6	23.2	47.0	15.9	65.7	46.1
	Mar.		89		0		3.4		0		39.3		42.7	
	Apr.		965	39	1.0	11.6	12.7	1.7	0	1.0	15.6	31.4	30.3	18.7
Satin leaf	Jan.		164		35.7		3.7			4.9				37.3
	Feb.		12		34.4								34.4	
	Mar.		115	199	67.0	30.1		4.0		34.2	1.7	1.0	63.7	69.3
Rose apple	May		1,935				1.9		20.4	1.3		3	21.7	
	June		8,615				7.3		24.2	13.9		1.3	43.8	
	July		831		7		14.8		21.8		5.6		62.9	
	Aug.		96				21.9		13.5		5.2		46.6	
French cherry	Jan.		205		2.9		1.5				5		4.9	
	Feb.		501		11.4		2.8				1.2		15.6	
	Mar.		77		57.9								57.9	
	Apr.		19	1,112	68.4	26.6		1.3		1.5		2	68.4	28.9
	May		6	256	30.9	28.8	16.7	8.3		5.1		4	66.7	42.8
	June		1,952			1.8		39.0		34.1		1		75.0
	July		15		12.0		34.3		2.9		2.9			81.9
	Sept.		322		15.9		11.8		18.0		6			65.4
West Indian medlar	Mar.		97		1.1						1.1		2.2	
	May		17		2.9								5.9	
	June		79											
Kamum	Jan.		314											3.8
	Dec.		578		1.3				2				5	
Yellow oleander	Jan.		80											10.0
	Feb.		154		1.0		1.0		13.3		13.0		30.4	
	Mar.		334						26.8				39.9	
	Apr.		7	35		31.4			2.9			17.1		71.4
	July		364		3		3.0		20.0		7.7		34.1	
	Aug.		107				1.0		11.9		11.9		24.8	
	Sept.		127						16.0		8.0		24.0	
	Oct.		25	35	4.0				26.0		8.0		34.0	
Carambola	Dec.		22		13.6				22.7		9.1		42.4	
	Sept.		6				16.7						33.4	

TABLE II.—Percentage of parasitism of larvæ of *Ceratitis capitata* in Hawaii in 1919 and 1920—Continued

Host fruit	Month	Number of larvae emerging during first 2 to 5 days.	Percentage of parasitism.									
			<i>Opus humilis</i>		<i>Diachasma tryoni</i>		<i>Diachasma fullawayi</i>		<i>Tetrastichus piffardianus</i>		Total.	
			1919	1920	1919	1920	1919	1920	1919	1920	1919	1920
Chinese orange.	Jan.	382			7.9		1.8		0.8		0.5	11.0
	Feb.	705	792	0.0	3.1	10.6	.7	1.0	1.7	1.8	1.0	14.0
	Mar.	440	129	1.0	20.0	0.0	.3	1.4	1.0		3.1	9.0
	Apr.	735	993	2.0	10.8	7.9	2.4	.7	1.7	.8	2.7	10.8
	May.	350	307	1.4	4.3	11.4	12.1		3.4		11.1	12.8
	June.	304	101	.3	14.6	6.9				5.4	1.0	14.4
	July.	60	279		4.7	10.0	10.4	3.8	4.3	10.0	1.0	23.3
	Aug.	159			8.2		4.4		.0		7.0	20.8
	Sept.	61			5.3		3.3					9.6
	Oct.	53	84	3.8	3.0		1.2		3.0		20.2	18.8
	Nov.	243	368	7.0	2.2	.8	9.5	1.0	20.1	3.3	5.4	7.7
Guava.	Dec.	245	147	4.5	3.7	1.7	4.8	4.7	4.8	2.0	12.2	11.8
	Jan.	1,125			8.7		3.9		5.2		7.9	21.7
	Feb.	240	1,139	2.9	10.0	1.4	13.8	1.4	1.2		18.1	8.7
	Mar.	1,213	181	1.9	11.3	4.0	2.0	.3	4.0	1.0	47.1	7.4
	Apr.	670	40		5.0	21.3		.3	5.0	11.5	35.0	35.1
	May.	1,115				27.5		.0		14.8		42.4
	June.	949			4.3		13.4		1.4		17.5	33.5
	July.	580	264	2.4	2.0	22.4	4.8	.7	2.0	18.0	31.9	44.1
	Aug.	202			.3		7.9		21.0		31.8	61.0
	Sept.	541	184		1.0	8.5	4.9	14.9	16.3	8.5	22.1	31.0
	Oct.	93	919		.5	3.7	14.8	14.0	14.8	9.7	10.9	28.0
Lemon.	Nov.	60	318				12.5		13.0	13.1	37.0	11.1
	Dec.	247	277	8.8		3.8	1.8	4.6	4.3	12.2	19.1	29.4
	Jan.	405			2.8		11.7		3.0		14.0	15.0
	Feb.	129			.8		.0					1.7
Gonionia.	Aug.	43					7.9					7.0
	Feb.	21			4.8				4.8		4.8	19.4
Orange.	Mar.	21	330		.0		1.2		3.0		7.0	12.4
	Apr.	22	142						2.8		7.7	10.5
	May.	5										
	Sept.	47									2.4	5.4
	Oct.	68						4.4		4.4		3.8
	Nov.	26								11.5		11.5
	Dec.	8	333		.3		9.4		1.3		20.4	24.3

Tables III and IV record the work of each parasite in all fruits collected over monthly and yearly periods. In Table III, the percentages of parasitism by *Diachasma fullawayi* for 1919 are typical of those for previous years. A comparison of those figures with percentages for 1920 in the adjacent column on the right again reveals the increase in the effectiveness of this parasite. In two months of 1920, January and August, its work exceeds that of any one of the other three parasites, and in six other months it was second in effectiveness. As indicated in a previous publication,<sup>4</sup> the ability of *D. tryoni* and *fullawayi* to destroy *Opus humilis* when they occur in the same host larva influences the amount of parasitism by *O. humilis* to a great extent. *O. humilis* has always been more abundant during the cooler months of the year, when the two species of *Diachasma* are less active and have a tendency to hibernate; and it decreases greatly in numbers during the warmer months, when it must contend with maximum numbers of *Diachasma*. A records of parasitism for 1916<sup>5</sup> the effectiveness of *O. humilis* during five months out of the year was greater than that of both species of

PENDERGTON, C. E., and WILLARD, H. F. INTERRELATIONS OF FRUIT-FLY PARASITES IN HAWAII. In Jour. Agr. Research, v. 12, no. 5, p. 285-296, PL. 12-13. 1918.  
PENDERGTON, C. E., and WILLARD, H. F. FRUIT-FLY PARASITISM IN HAWAII DURING 1916. In Jour. Agr. Research, v. 12, no. 2, p. 120-128. 1918.

*Diachasma*. In 1917 and 1918 <sup>6</sup> it was greater during two months; and in 1919 and 1920, for one month out of each year. The control exerted over *O. humilis* by *Diachasma*, reducing parasitism by the former as that of the latter increases, is clearly shown in Table IV. In 1915 *O. humilis* destroyed 31.5 per cent of all *Ceratitis capitata* larvæ under observation, or 83.1 per cent of the total parasitized larvæ. In 1920 its parasitism was only 9.4 per cent of all larvæ, and 18.1 per cent of the total parasitized larvæ. This great decrease in the numbers of *O. humilis* over a period of six years is due almost entirely to the cannibalistic habits of *D. tryoni* and *D. fullawayi*. *Tetrastichus giffardianus* probably destroys small numbers of *O. humilis*, as well as *D. tryoni* and *D. fullawayi*. Studies of the interrelations of these parasites <sup>7</sup> show that *T. giffardianus*, which does not resort to cannibalism, is capable of destroying opiine parasites occurring in the same host larva, probably by starvation. This parasite deposits about 10 eggs at one time in a single fruit-fly larva. The larvæ hatching from these eggs usually have no more than one opiine larva to contend with, and they absorb the food material of the host so rapidly that the opiine larva usually dies. Before death, however, the opiine larva often destroys many of the *T. giffardianus* larvæ, but no instance has been observed where the opiine larva survived, although such a case may be possible. Should *T. giffardianus* continue in numbers and effectiveness, it will doubtless cause a decrease in the numbers of the opiine parasites; and it will be interesting to note what effect the new proportions of parasitism will have on the amount of infestation by *C. capitata*.

The records of fruit-fly parasitism for 1919 and 1920 have shown several interesting facts in connection with the efforts to control the Mediterranean fruit fly by introduced parasites. The continued activities of these parasites during the past six or seven years, and the fact that they have destroyed approximately 50 per cent of the fruit flies developing during the past four years, have caused a noticeable decrease in the infestation of some of the most preferred host fruits of the fly. A great check has been exerted on the activities of *Opius humilis* by the two species of *Diachasma* until, in 1920, it was the least effective of the three opiines and parasitized a smaller percentage of larvæ than during any year since its introduction. *Diachasma fullawayi* and *Tetrastichus giffardianus* have increased greatly in value, and have proved their ability to attack fruit-fly larvæ in almost any fruit. While the use of parasites does not control the Mediterranean fruit fly in Hawaii, it has met with a large degree of success, as compared with other methods of combating this pest, and has decreased the infestation of many edible fruits to a marked extent.

<sup>6</sup> FLEMING, C. E., and WILLARD, H. F. WORK AND PARASITISM OF THE MEDITERRANEAN FRUIT FLY IN HAWAII DURING 1917. *In Jour. Agr. Research*, v. 14, no. 11, p. 695-716. 1918.

WILLARD, H. F. WORK AND PARASITISM OF THE MEDITERRANEAN FRUIT FLY IN HAWAII DURING 1918. *In Jour. Agr. Research*, v. 18, no. 8, p. 447-448. 1920. Literature cited, p. 426.

<sup>7</sup> FLEMING, C. E., and WILLARD, H. F. INTERRELATIONS OF FRUIT-FLY PARASITES IN HAWAII. *In Jour. Agr. Research*, v. 12, no. 5, p. 287-295, pl. 19-15. 1918.

TABLE III.—Total parasitism of all larvae of *Ceratitis capitata* collected in Hawaii during 1919 and 1920 (monthly averages)

Month.	Number of larvae.		Percentage of parasitism.										Total.	
			<i>Opus humilis</i> .		<i>Dacnusa brevia</i> .		<i>Dacnusa fulvipes</i> .		<i>Tetrastichus giffardianus</i> .					
	1919	1920	1919	1920	1919	1920	1919	1920	1919	1920	1919	1920	1919	1920
January.....	2,410	3,522	6.8	6.7	17.1	5.2	3.4	12.4	0.9	4.8	27.2	20.1		
February.....	5,812	2,113	19.4	10.0	11.8	7.7	1.9	4.4	2.4	10.0	37.5	38.7		
March.....	4,804	1,039	7.7	23.6	7.6	2.7	1.0	8.7	2.2	10.0	18.5	45.0		
April.....	2,877	1,023	4.9	13.0	13.3	3.9	2.2	3.6	8.0	3.8	26.4	26.3		
May.....	4,511	10,255	1.8	19.4	38.8	26.4	1.1	1.3	6.1	4.7	46.3	49.8		
June.....	4,100	10,512	5.1	7.6	34.3	24.8	1.3	11.4	6.3	1.3	40.0	48.0		
July.....	8,158	5,903	22.0	8.2	28.9	28.0	3.0	10.0	8.1	7.8	60.0	57.9		
August.....	7,638	5,684	7.4	8.1	8.3	13.7	1.0	21.1	15.5	10.5	37.2	57.0		
September.....	9,351	6,848	9.4	6.8	13.8	27.2	3.0	12.7	7.9	20.4	30.1	75.1		
October.....	10,676	8,104	6.3	2.0	14.7	26.8	2.6	8.5	6.1	7.1	26.0	54.4		
November.....	10,866	1,748	6.9	1.7	21.1	26.1	1.6	17.1	8.4	15.0	38.0	60.7		
December.....	5,994	1,145	11.0	5.8	33.3	4.4	0.0	9.7	9.9	18.4	55.6	33.0		

TABLE IV.—Total parasitism of all larvae of *Ceratitis capitata* collected in Hawaii from 1915 to 1920 (yearly averages)

Year.	Number of larvae.	Percentage of parasitism.				Total.
		<i>Opus humilis</i> .	<i>Dacnusa brevia</i> .	<i>Dacnusa fulvipes</i> .	<i>Tetrastichus giffardianus</i> .	
		Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
1915.....	28,010	31.5	0.3	5.9	0.2	37.9
1916.....	33,304	17.2	13.3	2.1	0.6	33.2
1917.....	72,139	12.7	20.3	7.3	7.2	47.5
1918.....	63,480	12.4	34.0	2.6	0.2	55.8
1919.....	75,406	9.4	19.0	1.6	7.6	38.2
1920.....	57,406	9.4	22.7	12.1	7.7	51.9





## ACID PRODUCTION BY RHIZOPUS TRITICI IN DECAYING SWEET POTATOES<sup>1</sup>

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### INTRODUCTION

Weimer and Harter<sup>2</sup> have recently shown that the carbohydrate losses from sweet potatoes undergoing decay by *Rhizopus tritici* Saito. exceed the weight of carbon dioxide evolved, and that the difference is considerably greater than the probable amount utilized in the construction of fungous tissue. They report an increase in the hydrogen-ion concentration of the juices of the decayed material, and indicate the probability of alcohol formation. They conclude:

That acids are produced in considerable abundance seems quite evident, and that alcohol is formed seems probable. The carbohydrates required for the manufacture of acids and alcohol together with that utilized directly in the production of fungous material will probably account to a large extent for the decrease in the sugars and starch which are not accounted for by the CO<sub>2</sub> evolved.

In the hope of securing additional information regarding the carbohydrate changes involved, particularly the identity of the acid or acids produced, the present writer, working with material courteously supplied by Weimer and Harter, has carried on certain additional experiments. The results, which are here presented, are confirmatory and show that the principal products of the fermentation are ethyl alcohol and acetic acid, but that formic acid, a trace of butyric acid, acetone, an unidentified aldehyde, and traces of at least two nonvolatile acids, one of which is lactic and the other probably succinic, are produced, and that a small amount of ammonia appears among the nitrogenous decomposition products.

### EXPERIMENTAL WORK

The decayed sweet potatoes employed resulted in most instances from artificial inoculation with *R. tritici*, but in a few cases the decay resulted from natural infection. Some tests were made in the early stages of decay while it was incomplete but actively progressing. In these cases only the softened portions of the tubers were employed. Most of the tests were made on material which had undergone complete decay in moist chambers in the incubator at 30° C. after which they were held for a period which varied from 1 or 2 to 18 or 20 days.

The results were substantially identical in all cases. For each trial about 10 tubers were placed in cotton cloth and subjected to heavy pres-

<sup>1</sup> Accepted for publication Mar. 15, 1923.

<sup>2</sup> WEIMER, J. L., and HARTER, L. L. RESPIRATION AND CARBOHYDRATE CHANGES PRODUCED IN SWEET POTATOES BY *RHIZOPUS TRITICI*. In *Jour. Agr. Research*, v. 21, p. 527-535. 1921. LITERATURE CITED, 254-537.

sure in a meat press. About 1100 cc. of brown liquid, acid to litmus, was thus secured. It was rendered alkaline with sodium carbonate and distilled with steam under approximately constant volume. After 100 to 150 cc. of distillate (which began to come over at about 92° C.) had been collected, distillation was interrupted and the material allowed to partially cool. An excess of phosphoric acid was then added and the copious precipitate of organic matter which appeared was centrifuged off, and the filtrate returned to the apparatus and distilled with steam at constant volume until two or three liters of distillate had been collected and the condensing vapors no longer gave an acid reaction to litmus. Distillation was then continued under diminishing volume until only 150 or 200 cc. remained in the distilling flask. This residue was allowed to cool and extracted with ether for several hours for the recovery of non-volatile organic acids.

The distillate obtained from the alkaline liquid possessed a neutral reaction and a strong odor of ethyl alcohol. It was inflammable, burning with a blue flame. It was found that 5 cc. yielded a very copious precipitate of iodoform when subjected to the usual potassium hydroxide iodine test. Heated with a few drops each of sulphuric and acetic acids, it yielded distinctly the odor of ethyl acetate.

With ammoniacal silver nitrate, 5 cc. of the distillate gave a heavy silver mirror. It also restored the color to magenta solution decolorized with sulphurous acid. It developed a pungent but not a lemon odor and a yellow color on boiling with sodium hydroxide. The color was at first clear yellow, then slightly cloudy, becoming clear again on further boiling, with the final development of reddish-brown, but not the yellow-orange color or the odors characteristic of either acetic or propionic aldehyde. The resorcin sulphuric acid tests for formaldehyde yielded a brown ring and a white precipitate, soon turning brown. The red color characteristic of formaldehyde did not appear. The gallic acid test with sulphuric acid was also negative for formic aldehyde.

The liquid gave a slight but distinct positive test for acetone with the Gunning iodoform test. A portion of the distillate, slowly heated with a distinct excess of Fehling's solution in a distilling flask and then distilled, yielded reduced copper in the flask and a distillate which reacted positively for alcohol but no longer gave the aldehyde or acetone reactions. The original distillate gave a positive reaction for ammonia with Nessler's reagent, and it was noted also in performing the Gunning acetone test that a black precipitate (nitrogen iodide), which disappeared on standing, formed immediately on the addition of iodine and before ammonia was used.

These tests show that the distillate contained a high percentage of ethyl alcohol, appreciable amounts of an unidentified aldehyde (not formic and probably not acetic or propionic), traces of acetone, and small amounts of ammonia.

The entire distillate from phosphoric acid was titrated with standard barium hydroxide and evaporated to dryness on a steam bath. It usually contained from 30 to 40 cc. of normal acid, though some of the freshly decaying samples yielded only 10 or 12 cc. The dried barium salts were extracted with 10 to 20 volumes of absolute alcohol for several hours with frequent trituration, filtered and washed with alcohol. The filtrate when evaporated yielded only a very small residue. When warmed with a drop or two of sulphuric acid the residue gave a rancid odor. The addition of a few drops of ethyl alcohol and further heating developed

an agreeable odor suggestive of pineapple, indicating the presence of butyric acid. The barium salts that were insoluble in alcohol were taken up in water, filtered free from the small amount of insoluble carbonates and decomposed by an excess of sulphuric acid. The barium sulphate was removed by filtration and the volatile acids removed by distillation from the filtrate. Tests for the identification of the acids were made on this distillate or on the sodium or barium salts obtained from it.

The reaction with ferric chlorid was positive for acetates and formates. With silver nitrate, a white crystalline precipitate, soluble in ammonia, was produced. On standing, or more promptly on heating, the precipitate acquired a dark tint, but the separation of metallic silver causing it was always small. Mercurous nitrate produced a white crystalline precipitate which also took on a grayish tint on standing or on heating. Mercuric chlorid gave a white precipitate of mercurous chlorid on heating, but its volume was very small for the amount of salt tested. Heated with sulphuric acid the salts gave off the odor of acetic and formic acid. Slight effervescence accompanied the reaction, yielding a gas which in carefully carried out tests could be ignited, burning for an instant with a blue flame. The ester obtained on heating the salt with sulphuric acid and alcohol suggested both methyl and ethyl acetates, in comparison with parallel tests on pure known salts alone and combined. The free acids were warmed at 45° C. with an excess of mercuric oxide, filtered, and the filtrate heated to boiling; a slight but distinct precipitate of metallic mercury was thrown down. White crystals of acetate of mercury appeared in the liquid on cooling. The sodium salt heated with paratoluidin and hydrochloric acid yielded an acid toluid which when purified and twice recrystallized melted at 145°-146° C., uncorrected. Acet-p-toluid melts at 148.2°C., corrected. A portion of the barium salts was purified by twice redistilling from strong sulphuric acid and again obtained as the barium salt. After recrystallization the barium content of the dehydrated product determined gravimetrically by precipitation with sulphuric acid was 53.53 per cent. A synthetic sample prepared from pure glacial acetic acid and the barium hydrate used in the work also yielded an average of 53.53 per cent of barium. The theoretical figure for pure barium acetate is 53.79 per cent barium.

From the foregoing tests it is evident that the volatile acid obtained was chiefly acetic but that a small amount of formic and a trace of butyric were also recovered.

The ether extract was examined for nonvolatile acids. On standing fine needle crystals appeared in the sirupy matrix remaining after evaporation of the ether. Water was added and the resulting solution titrated against phenolphthalein with standard barium hydrate, 50 to 60 cc. of *N/10* solution being required. The resulting barium salts were evaporated to dryness, triturated with 10 to 12 cc. of water, filtered, washed with a few cubic centimeters of water, and the filtrate and wash water treated with strong alcohol till the concentration was 90 per cent by volume. It was then placed in the ice box for a day or two, after which the precipitate was filtered off and washed with alcohol.

The filtrate was evaporated to a small volume, diluted with 20 cc. of water and carefully treated with *N/10* sulphuric acid, drop by drop, as long as calcium sulphate precipitated. The excess sulphuric acid added was removed by one or two drops of barium hydrate solution and the barium sulphate removed by filtration and washed. The filtrate and washings were evaporated on the water bath to a small

volume and tested for lactic acid by the Kelling ferric chlorid test and by Uffelmann's test, both of which gave positive reactions. The remainder of the liquid was placed in a test tube fitted with a conducting tube leading to a second test tube containing 1 cc. of water. On heating the material in the first tube it decomposed, giving off white vapors, which were absorbed by the water in the second tube. This material when boiled with 5 cc. of 10 per cent sodium hydroxid became first clear-yellow, then turbid, opaque and yellow-orange, giving a penetrating characteristic odor, thus confirming the previous tests for lactic acid.

The residue of barium salts of nonvolatile acids remaining undissolved after trituration with the 10 to 12 cc. of water, as indicated in the preceding paragraph, was dissolved in 50 cc. of hot water and decomposed with sulphuric acid. Any trace of excess acid was avoided. The material was concentrated on the steam bath to 10 cc., filtered into a weighed test tube, and evaporated to dryness on a steam bath while removing the vapors by an air current through a piece of glass tubing inserted in the neck. The dried residue of about .05 gm. was heated for 30 minutes with .3 gm. of p-toluidin in a bath at  $210^{\circ}\text{C}.$ , employing a reflux air cooler. After cooling, 5 cc. of 50 per cent alcohol were added, and then it was boiled, thoroughly cooled, and filtered. The crystals obtained were dissolved in 5 cc. of alcohol and recrystallized on a watch glass. White needle crystals, presumably succinotoluid, separated out, but the yield was almost microscopic in volume and too impure, as shown under the lens, to employ in a melting point determination. The precipitate filtered from the 90 per cent alcohol solution was also tested as outlined above for succinic acid but without positive results. The evidence obtained therefore indicates the presence of a trace of lactic acid and perhaps also succinic acid.

In order to make sure that the products identified were not the result of the activities of bacteria or other secondary organisms following the fungus, the work was repeated with substantially identical results, employing both sweet potato broth and sterilized sweet potatoes in flasks, as well as raw blocks of sweet potato cut from the tubers under aseptic conditions and placed in sterilized flasks. Raw sweet potato juice secured by grinding sound tubers of the same variety as those used in the inoculation experiments and subjecting the pulp to pressure in the meat press yielded a neutral distillate free from ammonia.

#### SUMMARY

It may be concluded that the fermentation produced in sweet potatoes decaying through the action of *Rhizopus tritici* is of the familiar alcohol-acetic acid type, in which, in addition to alcohol and acetic acid, much smaller amounts of formic, butyric, lactic, and succinic acids are found, as well as acetone and an unidentified aldehyde, and that ammonia is among the nitrogenous decomposition products.

# TEMPERATURE EFFECTS IN PLANT METABOLISM<sup>1</sup>

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## INTRODUCTION

In recent years considerable attention has been given by ecologists to climatic factors as determining plant distribution. Among the more recent publications in this field is the work of Livingston and Shreve (27).<sup>2</sup> The extensive observations of Bonnier (1, 2) upon anatomical and physiological modifications in plants of the same species, grown at different altitudes, are also noteworthy in this connection. This investigator has recently detected a complete change of variety in plants subjected for several years to such change of environment. To the physiologist, adaptations of this sort are explainable by the assumption that changes in the intensity of the various climatic factors disturb the chemical and physical equilibria which direct the growth process.

Followers of agricultural science are familiar with general relations between variations of climate and differences in the chemical composition of plants. Thus Hall (9, p. 83) states:

Even on the Rathamsted plots, where the differences in the supply of nutrients are extreme and have been accumulating for 50 years, the composition of the grain changes more from one season to another than it does in passing from plot to plot.

Beginning with the work of Richardson (31, p. 67; 32, p. 25) on analyses of grains from various regions of the United States considerable work has been done in this country upon the problem of environmental effects in the chemical composition of plants. Le Clerc (18, 19) has shown that the hot arid climate of Kansas is conducive to high protein content of wheat grain, irrespective of the types of soil tested by him. Richardson found no difference in composition of maize from different regions. No decided correlation between climatic factors and the composition of sweetcorn was found by Straughn and Church (38) in an investigation confined to the Atlantic Coast States. On the other hand, Wiley (41) found a distinct correlation between the sugar content of the sugar beet and the latitude of the State experiment stations which cooperated in his investigation. He concluded that temperature was the effective climatic factor in this case.

Apparently there exists an open question as to whether such climatic influences as have been mentioned here operate only upon the plant or act also indirectly through modification of the composition of the soil. Thus, while Lawes and Gilbert (17) found the proportion of grain in the wheat crop of Great Britain decreased by excessive rainfall, they attributed the effect partly to loss of nitrates from the soil by leaching. Furthermore, Gericke (7, 8) has shown that the protein content of wheat

<sup>1</sup> Accepted for publication Jan. 27, 1923. Published with the permission of the Director of the Wisconsin Agricultural Experiment Station, Madison, Wis.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 28-30.

grain can be increased by adding nitrates to the soil at a late stage of growth. He attributes the characteristically low protein content of wheat of the Pacific Coast States to deficiency of soil nitrates rather than to purely climatic influences. Finally, Lipman and Wayne<sup>2</sup> (20) have described rather profound effects of climate upon biological and chemical properties of the soil.

Recent investigations in this field have included attempts either to analyze climatic conditions more closely in relation to growth out of doors, or to control some of the climatic factors within greenhouses. Thus, Briggs, Kidd, and West (4) analyzing the data of Kreusler for the growth of maize, found the increase of weight per unit of leaf area better correlated with variations of atmospheric temperature than with variations of either illumination or rainfall. Their methods of treatment have been unfavorably criticised, however, by Fisher (5). In the case of peas grown in water cultures Brenchley (3) found the percentage rate of increase of the dry matter correlated with the temperature of the greenhouse at the foreperiod of growth, but with both temperature and sunshine thereafter. Walster (40) conducted sand cultures of barley in greenhouses controlled approximately to 15° and 20° C., but with the atmospheric humidity subject to influence by temperature changes. With a liberal supply of nitrates provided in the nutrient salts the plants grown at the higher temperature were excessively vegetative, while the other cultures supported normal culm formation. Under these conditions the leaves of the plants grown at the higher temperature were comparatively rich in soluble nitrogenous compounds, while they were relatively poor in sugars and other soluble carbohydrates. The reverse of this relation obtained with plants grown at the lower temperature. Differences were found in the distribution of various forms of phosphorus compounds in the plant tissue of the two types of cultures. The greatest quantitative difference found by chemical analysis, however, related to the polysaccharids. There were nearly 3 per cent more of these in the dry matter of the plants grown at 15° than in that produced at 20°.

The foregoing abstracts may serve to indicate the relative importance of temperature differences in climatic effects upon plant growth, as well as the apparently specific compositional response of the plant thereto.

It can be readily appreciated that such responses may bear important relations to disease resistance in the organism. As a matter of fact, plant pathologists have been giving increasing attention to environmental factors (12, 13, 14, 29, 39) in the investigation of disease relations. Furthermore, the work of Kraus and Kraybill (16) indicates important relations to fruitfulness of the ratio between nitrogen and carbohydrates of plant tissues. The possible practical importance of climatic modifications of plant composition thus becomes apparent.

#### EXPERIMENTATION

The more significant of the experiments to be described here were conducted in chambers especially constructed for regulation of atmospheric temperature and humidity.<sup>3</sup> Pending the development of these chambers the following preliminary test was made in greenhouses regulated roughly within different temperature ranges.

<sup>3</sup> Chambers for a similar purpose have been previously developed by Hottes, as mentioned by Felier (*loc. cit.* 1919); later developments have been described by Johanson (12).

RED CLOVER (*TRIFOLIUM PRAETENSE*) IN SOIL CULTURES WITHIN GREENHOUSES

The soil employed was Miami silt loam as described in a previous publication (10, p. 237). It was compacted moderately in glazed stoneware jars 21 cm. in diameter and 13 cm. deep (1-gallon crocks). These held conveniently 5 kgm. each of the air-dried soil. Two gm. of  $\text{CaCO}_3$  were mixed with each portion of soil. While filling the jars two of the cylindrical form of auto-irrigator (unground atmometer cups) introduced by Livingston (21, 22) were placed in the soil. These were connected with water reservoirs which could be adjusted vertically to regulate the plane of water in the soil of each jar separately.

On January 2, 1918, when the soil masses had attained a moisture content of 14 per cent (by weight), seeds from a vigorous commercial stock were sown in four jars. The jars remained in a greenhouse with a temperature range of  $15.5^\circ$  to  $21^\circ$  C. until the seedlings appeared, a period of six days.<sup>1</sup> Two of the jars were now transferred to another greenhouse with a temperature range of  $10^\circ$  to  $15.5^\circ$  C. The two pairs of cultures were placed in the same relative position in the southwest corner of the two greenhouses. A thermometer was plunged near the center of the soil mass in one pot of each pair and another was suspended with its bulb about 15 cm. above and midway between the two jars. The readings of these instruments were recorded daily, usually at about 4.30 p. m.

By occasional adjustment of the height of the water column connected with the irrigators the moisture contents of the soil in the several jars were increased and equalized. On January 14 the plane of soil moisture was 17.8 and 19.8 per cent in the cultures of the colder house and 19 and 20.3 per cent in those of the warmer one. These values approximate 40 per cent of saturation. The optimum content of this soil for red clover under similar greenhouse conditions, but in large containers, has been found to be 50 per cent of saturation.

The plants were reduced in number by removing the poorer individuals from time to time. This process was discontinued on February 10, when 8 plants per jar remained. After the plants reached considerable size and drew moisture rapidly from the soil the latter contracted, thus breaking contact with the irrigating cups. This caused variations in the plane of soil moisture among the several cultures.<sup>2</sup>

During the growth period the humidity of the air and approximate degree of illumination in the two houses were compared by means of the spherical form of the white and black atmometers devised by Livingston (22, 23, 24). In this case the water loss from the standard white porous clay instrument is employed as an index of the moisture deficit of the atmosphere, while the added evaporation from the blackened sphere serves as a comparative measure of light intensity.

The tops of the plants were harvested when the seventh and eighth leaves were emerging from the stools. This occurred on March 29 and April 13 at the higher and lower temperature ranges, respectively. On the former date the soil moisture was 10.8 and 15.4 per cent in the cooler house and 7.8 and 9.6 per cent in the warmer one. The value in the former case decreased to 7 per cent at the time of harvesting. After

<sup>1</sup> In view of the results of Kidd and West (25) relative to physiological predetermination, it would have been preferable to rear the seedlings at the temperatures in which the plants were to be reared.

<sup>2</sup> An improved form of irrigator which corrects this difficulty is described by Livingston (26).



drying at 100° C. the separate portions of tops were ground and subjected to chemical analysis by the official methods (42) commonly employed. The acid-hydrolyzable material is computed as glucose from the reducing power of the extract obtained with boiling 1.25 per cent  $H_2SO_4$  in determining crude fiber. Table I contains the data of climatic measurements and Table II shows the composition of the plants in this test.

TABLE I.—Climatic data of the environment of clover cultures

## SAME PERIOD AS OTHER CULTURES

House temperature.	Air temperature.			Total evaporation.		Ratio of evaporation.	Soil temperature.		
	Minimum.	Maximum.	Average.	White atmometer.	Black atmometer.	Black to white atmometer.	Minimum.	Maximum.	Average.
	°C.	°C.	°C.	C.	C.		°C.	°C.	°C.
Cooler.....	8.0	31.0	14.3	711	1,085	1.53	5.0	31.0	15.8

## FULL PERIOD OF GROWTH

Cooler.....	8.0	32.0	15.0	857	1,306	1.52	5.0	31.0	17.2
Warmer.....	13.3	29.0	20.6	1,047	1,420	1.36	11.0	32.0	20.2

TABLE II.—Composition of clover tops grown in different greenhouse environments

House temperature.	Dry matter of tops.	Yield of dry matter.	Crude protein.	Fiber extract.	Crude fiber.	Pentosins.	Poly-saccharids. <sup>1</sup>
	Per cent.	lin.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Cooler.....	(A) 21.5	12.75	18.5	5.1	15.0	9.2	10.5
	(B) 19.8	10.80	21.1	6.1	14.2	8.3	13.9
Average.....	20.7	11.78	20.0	5.6	14.6	8.8	12.2
Warmer.....	(A) 20.6	22.55	21.7	5.5	13.8	9.4	8.0
	(B) 22.7	9.45	22.5	5.4	14.8	9.6	8.6
Average.....	21.7	11.00	22.1	4.5	14.3	9.5	8.3

<sup>1</sup> Hydrolysis by boiling with 1.25 per cent  $H_2SO_4$  for 0.5 hour. Results are in equivalents of glucose.

Inspection of the climatic data shows that, with the exception of the maximal values which occurred toward the close of the experiment, a difference of about 5° C. was maintained in atmospheric temperatures. The average observed temperatures of the two houses approximated 15° and 20.6° C. for the full period of growth in each case. For the period of time when both pairs of cultures were growing simultaneously, the water loss from the white atmometer was 17 per cent greater in the warmer house than in the cooler one. This may be considered an index of the relative vapor pressure deficits of the atmosphere in the two cases. Apparently the relative humidity was nearly equal in the two greenhouses for the same relative humidity in both cases would bear the same ratio to each other as the total vapor pressures at saturation. Thus, with the latter values fixed at 12.2 mm. for 15° and 18.2 mm. at 20.5°

C. the evaporation value for any relative humidity below 100 per cent would bear the ratio 18.2:12.2 or 149:100. Hence, evaporation should be 49 per cent greater at the higher temperature than at the lower one. In the case of the black atmometers, which correspond more closely to plants than do the white ones as regards water loss when illuminated, it appears that the tendency to transpiration was about 31 per cent greater in the warmer house than in the cooler one. As will appear subsequently, this lesser difference of the black as compared with the white instruments is probably due to greater duration of sunlight in the cooler house.

Considering the increase of evaporation from the black atmometer over that from the white one as an index of the exposure to sunlight, it appears that the latter was appreciably greater in the cooler house than in the warmer one. This difference is probably due to the fact that the former house was on the western and the latter on the eastern side of a group of four parallel ranges of greenhouses. As a result, the cooler house received direct sunlight for a greater part of the day than the warmer one. The atmometric data indicate that the solar radiation was about 44 per cent greater in the former case.

The only significant difference in chemical composition between the plants grown at the two temperature ranges resides in the acid-hydrolyzable carbohydrates or polysaccharids. These compounds are included in the group of roughly defined hemicelluloses. There is no overlapping of the values for individual cultures at the different temperatures in this case, and the average value of the reducing sugars formed is about 4 per cent greater at the lower than at the higher temperature. Thus, a combination of relatively greater illumination, lower saturation deficit of the atmosphere, and lower atmospheric temperature was accompanied by increased storage of carbohydrates in the clover plant.

#### BUCKWHEAT (*POLYGONUM FAGOPYRUM*) IN SOIL CULTURES WITHIN CLIMATIC CHAMBERS

In view of the desirability of conducting plant cultures with variation of only one climatic factor, while limiting the others to as constant values as practicable, special chambers were constructed for this purpose. These were placed in the southern end of the greenhouse previously used for the higher temperature range. A section about 8 feet long at this end of the house was separated from the main portion by a partition of artificial boarding. The southern side of this partition was painted glossy white so as to reflect light into the climatic chambers which stood close by on the ends of the usual greenhouse benches. It was necessary to exclude sunshine from the culture chambers, for the radiation effects otherwise produced were uncontrollable. For this purpose curtains of bleached muslin were suspended from the ridge to the gutters of the greenhouse compartment and on the end of the house. These were pushed aside on cloudy days.

Plate 1, A, shows the climatic chambers in operation, together with portions of the humidifiers. The general arrangement consisted of humidifying chambers in which the minimum possible temperature imparted to the conditioned air current was limited by the temperature of the water supply from Lake Mendota.

As used, the temperature of the water was raised by electric heaters with thermostatic controls. On leaving the humidifier the air passed

through a cylindrical connection to the culture chamber. In this passage its temperature was raised by electric heaters which were controlled by a thermostat placed within the latter chamber. By this treatment the relative humidity of the air was reduced toward the desired value.

#### HUMIDIFIERS

The humidifiers were constructed from heavy galvanized iron sheeting after the plan of one described by Shamel (34). At the higher temperature 50 per cent greater length and capacity were provided than at the lower one. Each consisted of an upper tray 37.5 cm. wide and 5.6 cm. deep, resting upon a chamber 30 cm. in both width and depth. The tray consisted of troughlike sections 5 cm. in width and depth, so soldered together as to provide slits through which could be passed strips of toweling 30 cm. wide and 45 cm. long. The latter which were of coarse, open-meshed linen, were secured near one end to the bottom of the humidifying chamber by means of iron rods passed through loops in the toweling. The other end was drawn firmly through the slit above and fastened with brass clips. In this way one end of the toweling was bathed by water as it flowed through the tray and the other end was immersed in the overflow as it returned over the bottom of the chamber below. Baffle plates were arranged to direct the water and air currents completely in contact with the toweling. The water was heated by luminous radiator units of either 250 or 500 watts capacity. These were placed in copper cylinders which were sealed concentrically within iron ones, the water flowing between the two. A mercury thermometer which controlled these heaters was immersed in the water current near the entrance to the tray. Passing to the chamber below by a drainage tube, the water escaped to the drain through a siphon, thus preventing interference with its removal by the air current. The latter was provided by a No. 60 Buffalo forge blower operated continuously by a small electric motor. Air entered the humidifier at one end through a circular orifice 9 cm. in diameter and escaped to a heating cylinder through a similar orifice near the other end of the chamber. Here it was further heated by eight small cylindrical units of a capacity of 28 watts each, operated by a bimetallic thermostat suspended on the wall of the culture chamber. In all cases the thermostats were operated on 110 volts alternating current through pony relay instruments protected by ample resistance.

Heat insulation of the humidifier was provided by a blanket formed by supporting thin asbestos sheeting in cheesecloth. With 12 towels in the installation for lower temperature, air which was passed through the humidifier from the surrounding greenhouse at a probable rate of air replacement in the culture chamber of at least once in 5 minutes acquired a relative humidity of practically 100 per cent at 12° C. In the other installation, equipped with 18 towels, the relative humidity was adjusted to about 90 per cent at 18° C. The temperature of the lake water employed was about 6° in midwinter.

It was necessary to supplement the heaters of the humidifier at the higher temperature. This was done by coiling upon the water feed-pipe a section of resistance wire which gave approximately 500 watts continuous service outside the insulation. Through the further action of the heaters in the conduits connecting humidifiers with culture chambers, the conditioned air was delivered into the latter at about 17° C. and 70 per cent relative humidity in one case, and 22° and 78 per cent relative humidity in the other. Thus there was approximated an atmospheric

temperature difference between the two chambers of  $5^{\circ}$ , while practically equal saturation deficits were maintained in the two cases. A comparison of saturation deficit with relative humidity is given by Livingston (25). The necessary computations are as follows:

Temperature.	Vapor pressure at saturation. <sup>1</sup>	Vapor pressure at relative humidity of—		Saturation deficit (by difference).
		75 per cent.	78 per cent.	
$C^{\circ}$	<i>Mm.</i>	<i>Mm.</i>	<i>Mm.</i>	<i>Mm.</i>
17°	14.53	10.17	....	4.36
22°	19.83	....	15.47	4.36

<sup>1</sup>The values in this column are from Fowle (9).

As shown in Plate 1, B, where the black spores are conspicuous, the towel- ing was badly affected by molds. Addition of copper sulphate to the water stream proved ineffective after the organisms were established. From the experience of Morse with the destruction of copper-ferrocyanid membranes by molds (28, p. 533) there would seem to be little hope of avoiding difficulty by impregnation of the towel- ing with insoluble compounds of toxic elements. We are therefore substituting spray nozzles for humidification in future operation.

#### ILLUMINATION

Guided by exposure tests with photographic paper, additional shading was provided for the more westerly chamber, so as to equalize the solar radiation received by the two series of cultures, as indicated by the water losses from the black atmometers. The diminution of light intensity incident to the necessary shading of the chambers was partially compensated by placing a 500-watt, Mazda C, electric lamp over each. These were sufficiently distant to avoid serious heating effects and the light was concentrated upon the plants by conical reflectors. They were operated daily from about 5.30 p. m. to 9.30 p. m. and throughout cloudy days. Typical measurements of light intensity at the approximate level of the culture jars were obtained with a photometer. These appear in Table III.

TABLE III.—Photometric values of light intensity in foot candles

Character of day.	Location of test.			
	Shade of main house.	Isolated compartment south of chambers.	Within climatic chamber, lamp off.	Within climatic chamber, lamp on.
Clear .....	160	90	85	145
Cloudy .....	40	20	15	50

The beneficial effect of the artificial illumination was apparent in the growth response of the plants. Distinct etiolation of the latter became evident during early growth and before the lamps were installed.

With reference to the efficiency of the climatic apparatus as a whole, in view of the lack of refrigeration and other limitations it would be

manifestly unjustifiable to expect rigid control of atmospheric conditions by this installation. It was anticipated, however, that difference of evaporation could be restricted while maintaining a fairly constant temperature difference between the two culture chambers. It was evident that the plane of temperature in both chambers must be allowed to rise gradually as the season advanced into spring and the temperature of the lake water increased.

#### CULTURE CHAMBERS

The culture chambers were constructed from cypress 3 cm. thick in the form of four-paned window sashes as to sides and top. The sashes were set with glass of single thickness on both sides, thus providing heat insulation by an air space 1 cm. deep. The effective size of panes was 36.3 cm. square. Extra width of the bottom bars of the side sashes provided for isolation of a subchamber 10 cm. deep. Both floor and ceiling of this compartment were of 2 cm. pine boarding mortised to the sash bases. The culture chamber proper thus took the form of a cube with inside depth of 85.5 cm. It was painted glossy white throughout to promote reflection of light and heat.

A circular rotating table was provided in each chamber so as to facilitate uniform exposure of all cultures to varying degrees of temperature, humidity, and illumination. This was borne by a circular base of cast iron, 31.3 cm. in diameter, and projected upward in the form of a truncated cone. The base rested upon the bottom of the subchamber and its conical projection bore upon a ball bearing a cylindrical steel post 2 cm. in diameter and 10 cm. high. The latter was thus protruded through a central hole at the bottom of the culture chamber sufficiently to bear the rotating table. To it was fixed in the subchamber a wooden sheave 21.5 cm. in diameter and 2.5 cm. thick, by means of a central iron ring with set screw. The sheave was grooved for a small belt which passed through holes in the chamber wall to a reducing gear and motor outside. The table was composed of three pieces of cypress 2 cm. thick glued together to form a circular piece 75 cm. in diameter. It rested upon the supporting post through a centrally placed iron socket. Despite thorough painting it warped badly after some time. To avoid warping under these trying conditions, metal has been substituted for the wood.

One side of the chamber was supported upon hinges to allow access to both table and sheave. The conditioned air was conveyed from the heating cylinder outside to an opening beneath the edge of the rotating table by an extension of the sheet-iron cylinder projected diagonally upward on one side of the subchamber and converted to oval form as it opened into the culture chamber. To facilitate even distribution of the air upward, it was reflected beneath the rotating table by an arc of galvanized-iron sheeting erected close to the rim of the table from the base of the chamber. In addition, iron flanges were suspended radially at intervals from the bottom of the table. Air escaped from the chamber by a series of holes through the upper bars of the window sashes, whose total area was about 70 per cent greater than that of the intake.

#### SOIL CULTURES

On February 25, 1920, buckwheat seeds selected for uniformity of size from a Japanese variety were planted in 3.5 kgm. portions of Miami silt loam in earthenware jars (one-half gallon crocks). The soil was watered to 25 per cent of saturation, covered with paraffined paper, and placed

on the rotating tables. Each table bore six culture jars, a thermograph, a hygrograph, one white atmometer and one black one. The period of rotation was 40 seconds.

The seedlings appeared and were uncovered in 3 days at the higher temperature and one-half day later at the lower one. Thereafter the soils were brought to equal moisture content by weighing daily. On March 3 the number of plants per jar was reduced to 3 at the higher temperature, and a similar reduction was made at the lower temperature on March 5. On the latter date the plane of soil moisture was increased to 40 per cent of saturation. It was further increased to 50 per cent on March 19, and later reduced by two equal steps, on April 14 and 26, to 30 per cent. In the course of development aerial roots and red pigmentation appeared freely on the base of the stems for some distance

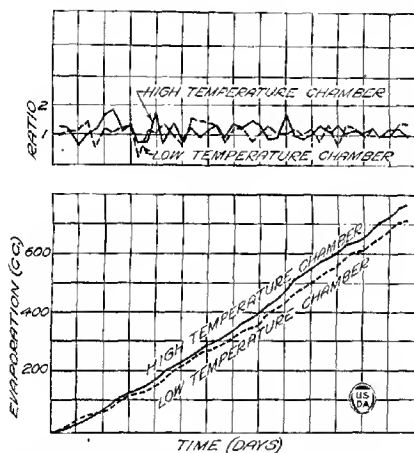


FIG. 1.—Relative evaporation (black atmometers) and relative solar radiation (ratio of evaporation between black and white atmometers). Climatic chambers, 1920.

above the soil. These features were especially prominent at the higher temperature.

On March 27, buds were unfolding at the higher temperature, although they were just appearing at the lower one. Several seeds had turned brown at the higher temperature on April 27, while those of the other cultures were still green. Photographs were taken on April 30. On May 10, the plants were harvested and separated into parts, as follows: leaf blades, petioles and stems, and seeds. These were dried at 100° C. and ground fine for chemical analysis. The data of climatic measurements and plant composition appear in Tables IV and V, while Plate 2, A, shows the appearance of the plants. Plate 3 shows the thermograph and hygrograph records. These are given for one week only, as it is hardly feasible to reproduce them in full. The graphs of figure 1 are constructed from the atmometric data.

Inspection of the climatic data shows that while the absolute extremes of temperature varied much more at the higher range than at the lower one, the daily average temperature varied only 4.5° in the former case and 3° in the latter. On the average, a difference of about 5.3° C. was maintained fairly continuously between the two chambers. The total evaporation during the growth of the cultures, as measured by the black atmometers, was only 6.5 per cent greater at the higher temperature than at the lower one. As measured by the white atmometers the difference of evaporation was 7.2 per cent. Had the mass of water remained the same at the former average temperature of 22.8° C. as at the latter average of 17.5° C, with the relative humidity at 70 per cent in the latter case, the saturation deficits would have been 10.32 mm. and 4.50 mm., respectively. Thus, if uncontrolled, the evaporation would have been 129 per cent greater at the higher than at the lower temperature. The normal tendency for difference in evaporation with uniform water supply was therefore greatly reduced in this experiment. As indicated by the ratio of water loss between the black and the white atmometers, the intensity of solar radiation was nearly the same in the two culture chambers.

TABLE IV.—Climatic records of plant chambers in experiment of 1920

Designation of temperature range.	Absolute maximum temperature.	Daily average maximum temperature.	Absolute minimum temperature.	Daily average minimum temperature.	Total evaporation standard black atmometer.	Ratio of evaporation black to white atmometer.
	°C.	°C.	°C.	°C.	Cc.	
High.....	33.0	25.0	10.0	20.5	769	1.083
Low.....	23.0	19.0	14.0	16.0	721	1.086

TABLE V.—Yield and composition of buckwheat, soil cultures of climatic chambers in 1920

Range of temperature (daily average).	Number of seeds at harvest. <sup>1</sup>	Dry matter of tissues.		Dry matter yield. <sup>2</sup>		
		Leaf.	Stem.	Leaf.	Stem.	Seed.
		Per cent.	Per cent.	Gm.	Gm.	Gm.
20.5 to 25.....	73	14.7	11.2	5.36	9.52	2.03
16 to 19.....	57	15.9	13.9	5.26	9.15	1.82

## Composition of dry matter.

Range of temperature (daily average).	Ether extract.			Polysaccharides. <sup>3</sup>			Nitrogen.		
	Leaf.	Stem.	Seed.	Leaf.	Stem.	Seed.	Leaf.	Stem.	Seed.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
20.5 to 25....	6.7	1.0	3.2	18.2	33.0	39.6	5.1	1.8	2.6
16.0 to 19....	8.5	.0	3.2	22.6	38.2	45.4	4.5	1.4	2.8

<sup>1</sup> Broken parts removed before harvest contained 2 immature seeds at higher and 12 at lower temperature.

<sup>2</sup> Dry weight of broken parts. Leaf at high temperature 0.41 gm., low temperature 0.46 gm.; stem at high temperature 0.32 gm., low temperature 0.30 gm.

<sup>3</sup> Hemicellulose of leaf and stem determined by hydrolysis with 4 per cent HCl 3 hours; starch of seed determined by digestion with saliva.

As regards the yield of dry matter, when allowance is made for the loss of one plant at the lower temperature it is apparent that this factor was virtually constant at the different temperatures. That such correction should not be proportionate to the loss of plants in proportion to soil space is indicated by the work of Stewart (37). It should be noted that the discrepancy in numbers of seeds will be greatly reduced when one adds to those matured others involved in immature broken portions of the cultures. As the data stand, the weight per seed was somewhat greater at the lower temperature. In view of the conditions just mentioned, this difference can hardly be considered important, because of the lessened competition for nutrients as compared with the cultures bearing more mature seeds.

The plants grown at the lower temperature had a higher percentage of dry matter than the others, especially in the stems. The ether extract was more abundant percentage in the leaves of plants grown at the higher temperature. This agrees with the deeper green color developed in this case, and is indicative of a relatively high chlorophyll content. The difference extended to the stems to a lesser degree, but disappeared in the seed, where the extract would be limited largely to true fats. A determination of the iodine numbers of the two fatty extracts of the seeds gave 55 per cent for the higher temperature cultures and 39.6 per cent for the others. However, the amount of material for analysis was too small to permit placing of emphasis upon these results.

Polysaccharids were more abundant throughout the plant in the cultures grown at the lower temperature, but the difference was somewhat less with the leaves than other tissues. In this connection, the work of Spoehr (36) should be noted. He found that with the approach of the dry season the cactus increased in pentosan content. The change was ascribed to a regulative mechanism for retention of water through production of polysaccharids of high capacity for hydration. Spoehr also concluded that a relatively high temperature (28° C.) produced the same effect, but his data are not so convincing as in the case of humidity effects. In an investigation of frozen peppermint Rahak (30) found evidence of increased esterification of menthol. A similar result was obtained by drying the plant tissue. Here, an extreme removal of water from liquid condition in the plant cells seems to have caused reversion of the familiar enzymic process of hydrolysis. It may well be questioned whether a similar effect would be likely to occur at temperatures much above freezing, but by hardening treatment with temperatures approaching freezing, Rosa (33) induced an increase of pentosan in certain plants.

It appears possible that the increase of polysaccharids observed in plants exposed to low temperature may bear some relation to disturbed equilibrium in the hydrolysis of these compounds. There are other possibilities, however, which should not be overlooked. One of these is the possible difference in net temperature coefficients for the synthesis of polysaccharids and of proteins in the plant. Another, and one rather more plausible than the others, is the possibility of limitation of polysaccharid storage due to consumption of sugars by increased respiration at higher temperatures. In this case the tissues would be expected to become richer in nitrogen, as is found by analysis.

With the exception of the seed, the nitrogen content of the several tissues of these buckwheat plants varied inversely as the polysaccharid content, but, even when expressed as equivalents of protein, they do



not compensate the variations of carbohydrates. In these cultures the modifying effect of varying temperature has been relatively free from disturbance by variations of either solar radiation or atmospheric humidity. Under these conditions an increase in polysaccharids has attended a decline of temperature value.

#### WATER CULTURES

Water cultures of buckwheat were conducted for a time parallel to the progress of the cultures just described. Seeds from the source previously used were suspended upon Shive's best solution for early growth of this plant, diluted to one-tenth the usual concentration. A group of seedlings were started in this manner in each chamber on March 15. Three seedlings were set up in each of four culture jars at the higher temperature on March 22, still employing one-tenth the usual concentration of Shive's solution. Two days later the seedlings were large enough at the lower temperature to be similarly transferred. At this time all of the nutrient solutions were made up to the usual concentration. On April 7, the third leaf was appearing in plants at the higher temperature, while the second leaf was just appearing in the other case. The leaves were greener and bases of the stems redder in the former case. Buds appeared on April 15 at the lower temperature and two days later at the higher one. On April 21, the solutions were changed to Shive's best proportions of salts for the last period of growth (35). The plants were harvested on April 26. At this time curling of the leaves and other indications of abnormal growth were becoming conspicuous, especially at the lower temperature. Only seven plants appeared reasonably normal in the latter case, and hence the seven best plants were selected from each series. The data of yield and composition appear in Table VI.

With the exception of the length of the tops, the physical measurements show little difference in the effect of the two temperature ranges upon the development of the plants. The slight difference in polysaccharid content varies in the same direction as with the soil cultures—that is, it was greatest at the lower temperature.

#### BUCKWHEAT IN SAND CULTURES WITHIN CLIMATIC CHAMBERS

Buckwheat was grown in sand cultures in the climatic chambers in 1921. This was for the purpose of avoiding possible modifying effects of temperature upon the fertility of soil, through action upon the soil organisms and in other ways. With the exception of using sand in place of soil and planting about two weeks later in the year, the experiment was conducted in essentially the same manner as the preceding one.

TABLE VI: Growth measurements and composition of buckwheat water cultures of climatic chambers in 1920

Designation of temperature range.	Minimum length of tops.	Maximum length of roots.	Weight of dry matter. <sup>1</sup>	Composition of dry matter.	
				Blow extract.	Polysaccharids. <sup>2</sup>
	Cm.	Cm.	gm.	Per cent.	Per cent.
High .....	48.8	10.6	0.72	4.4	24.7
Low .....	31.8	18.5	1.82	4.6	25.4

<sup>1</sup> Total weights, including discarded plants, 0.11 gm. at high temperature and 1.19 gm. at low temperature.  
<sup>2</sup> By boiling with 4 per cent HCl 3 hours after extraction of sugar and dextrins.

The sand was a mixture of 1 kgm. 100 mesh and 2 kgm. 50 mesh angular grains of quartz for each culture jar. It was rendered free of nutrients by extraction with hot 20 per cent  $\text{HNO}_3$  and thorough washing, followed by leaching with lime water. The mixture had a water holding capacity of 42 per cent.

After standing in the climatic chambers a sufficient period to insure temperature equilibrium, the 12 jars of sand were planted on March 12, and watered to the extent of 10 per cent of the sand by weight. Radicles appeared above the sand on March 14, at the higher temperature and one day later in the other case. On the latter date the covers were removed and there were added 0.2 gm.  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  and 0.4 gm.  $\text{KNO}_3$  per jar, in solution.

The water plane of the sand was raised to 13.5 per cent on March 17. By March 21, higher percentage and vigor of germination were apparent at the lower temperature. On April 2, 16 of the 18 plants in this case were expanding the first true leaves, while only 8 plants had reached this stage at the higher temperature. A further addition of nutrients was applied per jar on April 4 as follows: 0.1 gm.  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.2 gm.  $\text{KNO}_3$ , and 0.01 gm. ferric citrate. The newer leaves were pale green at this time, and noticeably mottled at the higher temperature. On April 8, the moisture plane of the sand was raised to 17 per cent.

Buds appeared in both series of cultures on April 14, but they were more numerous at the higher temperature. On this date each jar received the following nutrients: 0.3 gm.  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  and 0.6 gm.  $\text{KNO}_3$ . The final application of salts per jar was made on April 25, as follows: 0.6 gm.  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 1.2 gm.  $\text{KNO}_3$ , 0.39 gm.  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ , 0.075 gm.  $\text{KH}_2\text{PO}_4$ , 0.075 gm.  $\text{NaCl}$ , and 0.015 gm. ferric citrate. At this time the plants at the lower temperature were uniform in size, while the other series was irregular in this respect. On April 29, the water supply was raised to 20 per cent of the sand, or about optimal for the plants.

Conspicuous differences in the reproductive phase of growth soon appeared. Thus on May 2 all of the plants at the lower temperature were in full bloom, while only a few plants were in bloom at the higher temperature. Thickening of the stems was rather prominent in the latter case. On May 6 several seeds were developed to considerable size at the lower temperature, while only one seed had appeared at the higher temperature by May 10.

After taking photographs on May 16, the cultures were harvested. Plants with only two true leaves or decidedly pale in leaf color were rejected. There remained 16 plants at the lower temperature and 13 at the higher. These were separated into leaves and stems, excluding the seed parts. The data of climatic factors and chemical analysis are assembled in Tables VII and VIII. The appearance of the plants is shown in Plate 2, B, while Plate 4 shows a portion of the climatic records. Graphs constructed from the atmometric data appear in figure 2.

Comparison of the climatic data of this experiment with that of 1920 was less satisfactory in the present case. This is to be ascribed largely to less effective functioning of the humidifiers, due to deterioration of the toweling. In future development of the apparatus humidification will be accomplished by means of spray nozzles. The variation of the lower temperature range here was greater than that of the upper range, while the reverse was true in the earlier experiment. As a general average, the temperature was maintained at about  $5^\circ\text{C}$ . between the temperature planes here, but the average planes of operation were about

2.5° higher than in the earlier experiments. The attempt to check evaporation at the higher temperature was overdone, so that evaporation, as measured by the black atmometer, was 10 per cent less than at the lower temperature. The ratio of water losses from the black and

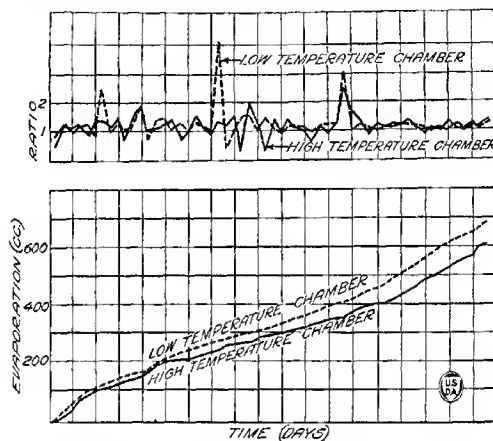


FIG. 2.—Relative evaporation (black atmometers) and relative solar radiation (ratio of evaporation between black and white atmometers). Climatic chambers, 1921.

white atmometers indicate practically equal illumination in the two culture chambers.

TABLE VII.—Climatic records of plant chambers in experiment of 1921

Designation of temperature range.	Absolute maximum temperature.	Daily average maximum temperature.	Absolute minimum temperature.	Daily average minimum temperature.	Total evaporation—standard black atmometer.	Ratio of evaporation—black to white atmometer.
	°C.	°C.	°C.	°C.	G.	
High.....	34.0	28.2	18.5	23.2	670	1.085
Low.....	30.0	23.3	14.0	16.9	678	1.085

TABLE VIII.—Yield and composition of buckwheat, sand cultures of climatic chambers in 1921

Range of temperature (daily average).	Number of seeds at harvest.	Dry matter yield <sup>1</sup>		Ether extract.		Composition of dry matter.			
						Polysaccharides <sup>2</sup>		Insoluble nitrogen <sup>3</sup>	
		Leaf.	Stem.	Leaf.	Stem.	Leaf.	Stem.	Leaf.	Stem.
°C.		Gm.	Gm.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.
23.2 to 28.2.....	0	0.47	0.66	6.6	4.5	18.3	14.8	2.3	0.7
16.9 to 24.5.....	6	.30	.79	7.1	4.1	17.9	19.6	2.5	.8

<sup>1</sup> Thirteen plants at high temperature, 16 at low temperature.

<sup>2</sup> Determined by boiling with 4 per cent H<sub>2</sub>SO<sub>4</sub> for 2.5 hours after extraction of sugars and dextrins.

<sup>3</sup> Determined on the residue from acid hydrolysis with one-half the acid extract added.

When the relative numbers of plants selected are considered, it appears that the development of dry matter in the leaves was appreciably lower at the lower temperature. The generally greater development of height of plants at the higher temperature is shown in Plate 2, B. Only well-filled and apparently normal seeds are considered in the data. On this basis there was a marked deficiency of the reproductive function at the higher temperature. This merits attention in connection with the chemical composition of the plants. With regard to the latter factor, the only distinct difference is in the polysaccharid content of the stems. In this respect the experiment agrees with the one conducted upon soil in 1920, in that the plants grown at the lower temperature contained about 15 per cent more of this constituent. It seems desirable to suggest that, in connection with the limited general development of these cultures, those at the higher temperature may have been unfruitful because of an unfavorable balance between nitrogen and carbohydrate content, according to the conclusions of Kraus and Kraybill (16).

#### SUMMARY

(1) A brief digest of the literature has shown variations of form and composition of plants in response to variations of climatic factors. In certain cases the decrease of temperature appears to have been specifically associated with increase of polysaccharids in the plants. The importance of these relations to problems in physiology is mentioned.

(2) Red clover (*Trifolium pratense*) grown in two greenhouses at 15° and 20.6° C. average temperatures, with constant soil water supply, but with 47 per cent excess of evaporation at the higher temperature and 44 per cent excess of solar radiation at the lower one, contained about 4 per cent more of polysaccharids in the tops of the plants grown at the lower temperature than in the other case. The crude protein content of the plants was least at the lower temperature, but not in proportion to the difference of polysaccharids.

(3) Chambers for the control of atmospheric temperature and humidity are described.

(4) Buckwheat (*Polygonum jagopyrum*) grown in soil cultures with a uniform supply of soil moisture at average atmospheric temperatures of 17.5° and 22.8° C., with evaporation 7.2 per cent greater at the higher temperature than at the lower one and with the reinforced solar radiation 3.6 per cent greater in the latter case, contained 5.8 per cent more starch in the seeds and 5.2 per cent more polysaccharids in the stems at the lower, as compared with the higher, temperature. The nitrogen contents of the stems and leaves varied inversely as the polysaccharid contents, but not proportionately so.

(5) Buckwheat (*Polygonum jagopyrum*) grown in sand cultures with uniform supplies of water and nutrient salts at average atmospheric temperatures of 20.1° and 25.7° C., with evaporation 10 per cent less at the higher temperature than at the lower one, and with reinforced solar radiation equal in the two cases, contained 4.8 per cent more polysaccharids in the stems at the lower temperature than at the higher one. The plants grown at the higher temperature produced no seeds of normal appearance.

(6) From the results herein presented, it appears that independent of its indirect effects through modifying the soil and independent of certain variations of atmospheric humidity and total magnitude of exposure to

solar radiation, atmospheric temperature modifies the percentage of polysaccharids in tissues of the plants here tested.

(7) Suggestions are offered as to the possible mechanisms by which the temperature effect here observed may be consummated.

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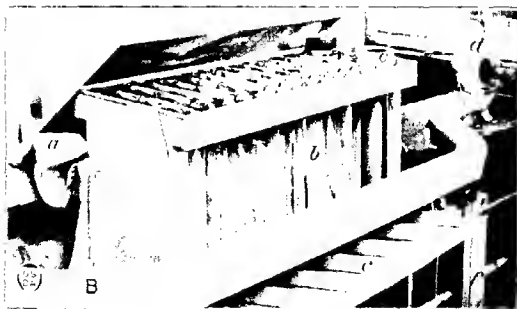
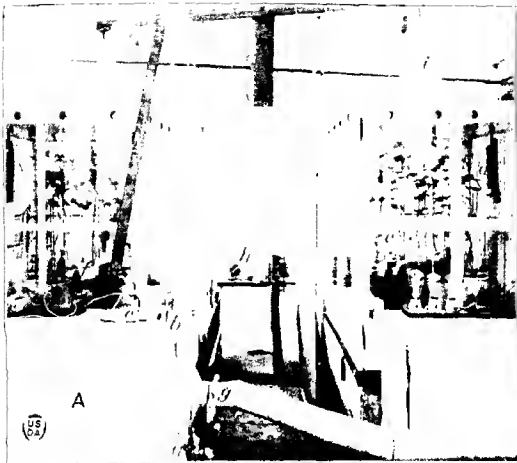




PLATE 1

- A. Climatic chambers in operation.
  - a. Water supply to humidifier.
  - b. Heating units of humidifier.
  - c. Mercury thermostat of humidifier.
  - d. Bimetallic thermostat of culture chamber.
  - e. Atmometer on rotating table.
  - f. Hygograph and thermograph on rotating table.
  - g. Air conduit with gate.
  - h. Motor and reducing gears belted to rotating tables.
  - i. Electric lamps.
- B. Humidifier.
  - a. Asbestos blanket.
  - b. Toweling, showing brass clips in tray above.
  - c. Concentric cylinder to contain heating units for water supply.
  - d. Fan supplying air.
  - e. Front flanges directing air current.
  - f. Escape to drain.

PLATE 1



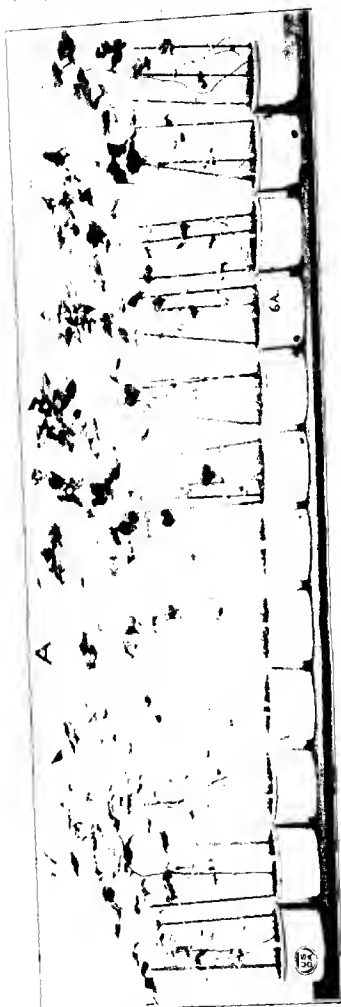


Fig. 1. The relief from the tomb of the Prince of the West.

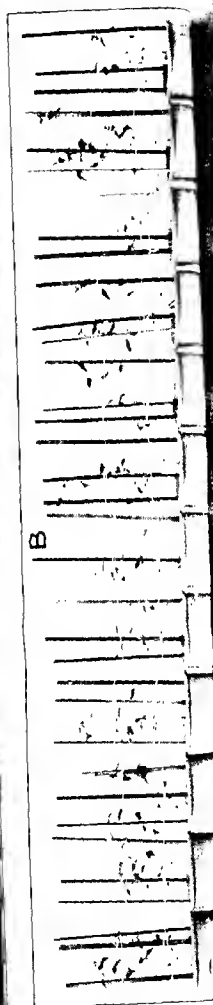


Fig. 2. The relief from the tomb of the Prince of the West.

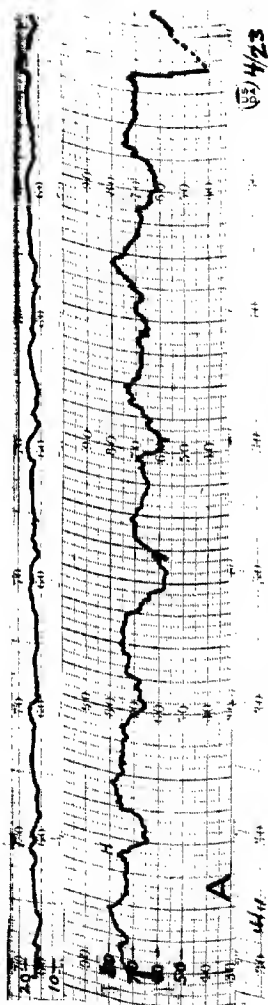
PLATE 2

- Cultures of buckwheat on soil in climatic chambers in 1920.  
Six left-hand cultures at lower temperature.  
Six right-hand cultures at higher temperature.
- Cultures of buckwheat on sand in climatic chambers in 1921.  
Six left-hand cultures at lower temperature.  
Six right-hand cultures at higher temperature.

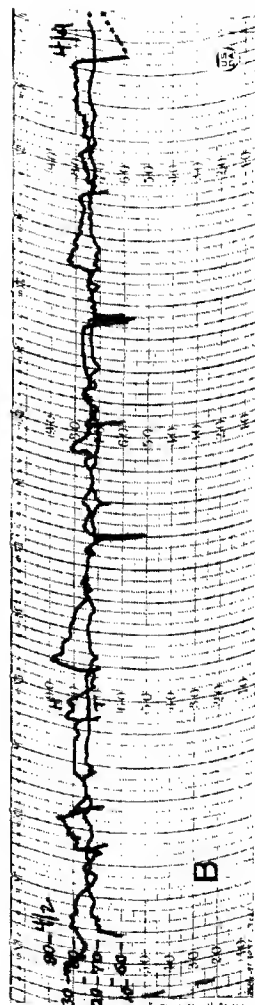
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PLATE 3

- A.- Climatic records, low temperature chamber, week beginning April 2, 1920.  
B.- Climatic records, high temperature chamber, week beginning April 2, 1920.



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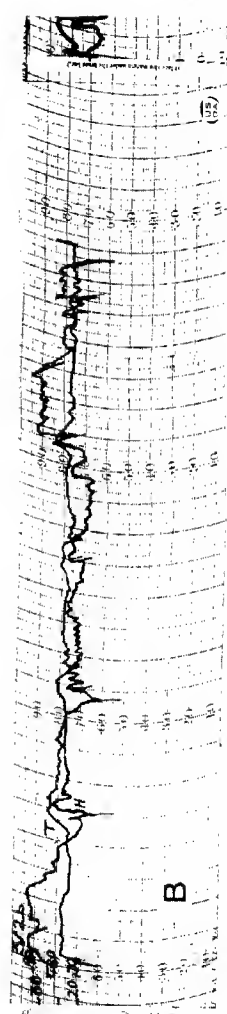
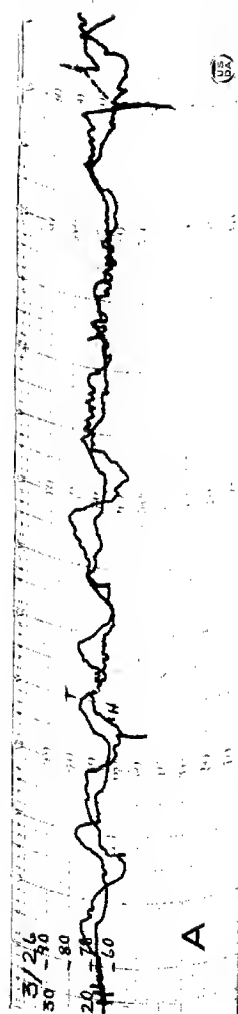


PLATE 4

- A.**—Climatic records, low temperature chamber, week beginning March 26, 1921.  
**B.** Climatic records, high temperature chamber, week beginning March 26, 1921.





# PLATYGASTER VERNALIS MYERS, AN IMPORTANT PARASITE OF THE HESSIAN FLY<sup>1</sup>

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## INTRODUCTION

In the fall of 1914, W. R. McConnell and P. R. Myers undertook an exhaustive study of the Hessian fly (*Phytophaga destructor* Say) at Hagerstown, Md., with particular reference to its parasites. Since that time more or less consecutive records have been kept of the occurrence of one of these parasites, *Platygaster vernalis* Myers,<sup>3</sup> throughout the eastern wheat-growing region. In 1917 the laboratory at Hagerstown was transferred to Carlisle, Pa., and in this year the writer joined in the investigations. During the course of these studies *P. vernalis* was first recognized and described by Mr. Myers,<sup>4</sup> but this parasite was not studied intensively until 1918, when the writer undertook to discover the details of its life history. This work, with many interruptions, has continued to date. The present paper summarizes the data collected on this parasite since 1914.

## ECONOMIC IMPORTANCE

From the standpoint of economic importance *Platygaster vernalis* stands first among the many species of parasites that normally attack the spring generation of the Hessian fly in the Middle Atlantic States. Percentages worked out for various species of Hessian fly parasites have shown that *P. vernalis* is more effective than any other species attacking the spring generation of the fly in this region.

TABLE I.—Percentage of Hessian flies killed by *Platygaster vernalis* for the years 1915 to 1920, inclusive, together with the total puparia examined in order to obtain these data, with the average and total for the entire period

Year.	Percentage killed by <i>vernalis</i> .	Number of puparia ex- amined.
1915.....	40.10	2,582
1916.....	15.53	2,285
1917.....	15.73	2,143
1918.....	19.00	6,930
1919.....	24.68	2,297
1920.....	27.34	2,419
Average.....	23.89	18,656

<sup>1</sup> Accepted for publication Aug. 15, 1922.

<sup>2</sup> The writer wishes to express his appreciation of the assistance rendered by the late W. R. McConnell and by P. R. Myers in contributing useful suggestions during the progress of the work and in helping to rear and determine much of the material used; he also wishes to thank Dr. R. W. Lohy for helpful criticisms, Messrs. R. M. Fouts and H. D. Smith for the determination of many of the parasites, and Miss Esther Hart for the drawings of the adult, head, and antennae.

<sup>3</sup> (Order Hymenoptera, superfamily Serphoidea, family Platygasteridae).

<sup>4</sup> MYERS, P. R. A NEW AMERICAN PARASITE OF THE HESSIAN FLY (*PHYTOPHAGA DESTRUCTOR* SAY). In Proc. U. S. Nat. Mus., v. 53, p. 255-257. 1917.

An average of 23.89 per cent of the spring generation of the host, destroyed annually by *Platygaster vernalis* throughout this territory. This statement is based on the examination of 18,656 puparia collected during a period of six years (1915 to 1920, inclusive) from 39 well-separated localities ranging from Montoursville, Pa., on the north, to Staunton, Va., on the south. Table I gives the percentage for each of these years and the average for the period.

#### DISTRIBUTION

*Platygaster vernalis* has been found throughout the eastern wheat-growing region as far north as  $43^{\circ} 33'$  and as far south as  $37^{\circ} 50'$  north latitude. Considerable material collected at Evans Mills and Theresa, N. Y. (a little north of the forty-fourth parallel), revealed no *vernalis* present. No abundance of *vernalis* was found farther south than Staunton, Va., a short distance north of the thirty-eighth parallel. This species was not found in material collected at Lexington, Va., about 35 miles southwest of Staunton, but one specimen was reared from material taken at Buchanan, about 20 miles southwest of Lexington, latitude  $37^{\circ} 50'$ . In the Middle West *P. vernalis* has been found in abundance at Wanatah, Ind., and records have been made of its occurrence at Niles, Mich., Strongsville, Wooster, Troy, and Columbus, Ohio, and Charleston, Mo.

#### THE EGG

The egg is highly refractive, claviform in shape, and before oviposition measures about 0.07 millimeter long by 0.016 millimeter wide. (Pl. 1, A, B.) A minute projecting piece of membrane may sometimes be found at the swollen extremity. Immediately after oviposition the main body of the egg (Pl. 1, B) is usually found dilated to about twice the width mentioned. Plate 1, A, shows a camera-lucida sketch of an egg freshly removed from the ovary, and Plate 1, B, represents an egg immediately after it was oviposited into the host egg.

The egg never develops except in the midintestine of the host. Surrounded by the chyle of the stomach, it is tossed about by peristaltic action. Plate 1, C, shows a Hessian fly larva in longitudinal section with a single *vernalis* egg about 22 days old submerged in the chyle in the midintestine.

The germ cell of the egg, instead of developing as a single embryo, as is the case with most insects, gives rise to several embryos. The nutritive plasma also develops precociously. Plate 1, D, shows a parasite body about 11 days old in sagittal section with four embryos in the blastul stage of development. Paranuclear masses may be seen scattered irregularly about in the surrounding plasma. Plate 1, E, shows a parasite body containing eight embryos that are much further advanced. Each embryo is surrounded by an individual membrane and the surrounding plasma is gelatinous in consistency. Paranuclear masses are still present.

The illustration shows the entire mass somewhat flattened out. In the host's stomach it tends to assume a spherical shape, but its plastic nature permits it to be compressed into various shapes by the peristaltic action.

The number of embryos found to develop from a single egg range from 2 to 12.

<sup>1</sup> The outlines of the sketch were made by camera lucida immediately after the embryonic mass had been taken from the host body and brought into normal salt solution. Later the embryonic mass was stained in picoscarmine to bring out the cell structure, and greater details of the sketch were taken from these stained and mounted embryos.

## THE PRIMARY LARVA

In general outline the primary larva (Pl. 2, A) is elongate oval, with length of body about three times its width and with a very slight taper toward the caudal end. Both extremities are bluntly rounded, and before the larva has become very much inflated with food a broad, deep constriction is evident on each side slightly posterior to the mouth. This constricted appearance is due to the greatly enlarged and projecting bases of the mandibles.

The mandible (Pl. 2, B) is noticeably long, being a little over one-third the width of the head. Measurements of single mandibles from four larvae show an average length of 0.073 millimeter. The mandible is wide at the base but tapers to a sharp extremity, with the distal third slightly curved. Several long, closely adhering spines are discernible along the curved portion and at the extremity. The entire mandible is nearly colorless and comparatively fragile. The mouth consists of a small transverse aperture, capable of being opened and closed by motion of the superior lip.

The larva is usually sufficiently transparent to disclose under proper magnification certain outstanding features of the internal anatomy, such as the cells of the stomach wall and epithelium. Some of the cellular structures of the internal anatomy, including the stomach wall and proctodaeum, are illustrated in Plate 2, A. The details of cell structure were taken from microtomic sections.

When the larva has developed sufficiently to feed, a movement of the labrum begins, thus producing a suction whereby the surrounding liquids or adjacent tender tissues are ingested. After freeing itself from the surrounding embryonic mass the larva first imbibes the green chyle from the host's stomach and soon ingests particles of the stomach wall itself. While it may possibly secrete juices which have a softening effect on surrounding tissues, nevertheless undissolved particles of host tissue within the stomach of the parasite have been clearly seen, and in one case part of the stomach tissues of the host was observed protruding from the parasite's mouth.

Frequently the number of young larvae found in a single host much exceed the number that ever reach maturity. Single hosts have been found to contain 21, 27, 32, 34, and 40 young larvae. In several instances 1 or 2 of such larvae were found in a stunted condition while the others in the same host were normal. In one host a single dead larva was discovered, while the others appeared normal and healthy. In another case 5 partially developed young larvae (2 of these exceptionally large ones), and 4 very small, poorly developed ones were found. At times all the young larvae were found dead within the host. Where this occurred the cause frequently was found to be hyperparasitism by undetermined chalcidoids.

## THE MATURE LARVA

The mature larva (the lateral aspect of which is shown in Pl. 2, C) is about 1 millimeter long by 0.5 millimeter thick. It is white, ovoid, bare of setae, and with 11 clearly defined body segments. Spiracles are present on the second and third thoracic segments and second abdominal segment only. In the first abdominal segment, instead of an external spiracle, a large discoidal body occurs under the cuticle at the terminus of the lateral tracheal branch of this segment. The mouth (Pl. 2, D) is a small

transverse orifice, when closed appearing as a somewhat crescent-shaped slit and when open forming an oval-shaped aperture. In the process of feeding, the superior lip is moved toward and away from the inferior lip by strong radiating muscles. The inferior lip is slightly thickened along its rim. On each side slightly posterior to the mouth is a small, distinctly curved mandible. (Pl. 2, E.) The mandibles are widely separated from each other, distinctly chitinated, and less than half the length of the mandible of the primary larva. The average length of 10 mandibles from 10 mature larvae was found to be 0.03 millimeter. Slightly caudad of the mandibles is a pair of faintly chitinated maxillae (Pl. 2, F.), which are rather broad at the base and taper slightly to a blunt point. The maxillae are slightly longer than the mandibles, with the inner side a little shorter than the outer.

During this stage of the parasite, the remainder of the host is consumed as far as the cuticula, which is left to inclose the cocoons that subsequently are formed.

#### THE COCOON

The cocoon (Pl. 3, A) is broadly ellipsoidal and of a flexible, smooth, shiny, yellowish brown consistency. The cuticula of the host is left intact, but adheres so tightly to the cocoons as to appear as part of them and doubtless serves as a protection to them. In Plate 3, A, the external spatula of the host may be seen adhering at one extremity. From 3 to 13 such cocoons have been found in single hosts, and the examination of 103 hosts showed an average of 7.91 cocoons per host. The host invariably formed its brown puparium case before being killed by the larvae of *Platygaster vernalis*, thus providing the hibernating parasites an additional protection.

#### THE PUPA

The pupa (Pl. 3, B), when first formed within the cocoon, is white, but the compound eyes soon darken and gradually the entire body turns a shiny black with the exception of the thin integument between the abdominal plates. The pupal stage is shorter in duration than either the larval or the adult stage.

#### THE ADULT

##### DISTINGUISHING MORPHOLOGICAL CHARACTERISTICS

The adult (Pl. 4, A) is from 0.7 millimeter to 0.9 millimeter long with shining black body. Certain distinguishing characteristics are as follows:

Head quadrate, about as wide as thorax; face convex; ocellus, vertex, and face distinctly transversely rugulose (Pl. 4, B); antenna with base of scape black, second joint of the flagellum in male distinctly curved and larger than that of the female (Pl. 4, C and D); scutellum laterally margined; legs entirely piceous; tarsi fuscous; wings 2½ to 3 times as long as abdomen; ovipositor (Pl. 3, C and D) straight, slightly enlarged and blunt at apex. Plate 3, D, shows the sheath, gorgonet, and stylet of the ovipositor. The ovaries are also characteristic of the species, being nearly spherical, with short thick oviduct about as long as the diameter of the ovary. A single ovary is shown in Plate 3, E.

#### PARTHENOGENESIS

Experimental rearings have shown that parthenogenesis may occur in *Platygaster vernalis*. In one experiment seven unfertilized females were put under a cloth-topped glass cylinder on potted wheat plants bearing eggs of the Hessian fly on their leaves. The Hessian fly larvae which

developed from these eggs were killed on different dates and sectioned. Three such larvæ killed at the end of 26 days contained normally developing *vernalis* embryos; two larvæ killed at the end of 43 days disclosed the presence of healthy, mature *vernalis* larvæ; and two killed at the end of 58 days contained *vernalis* cocoons. Two similar cages showed the development of healthy *vernalis* embryos.

#### SEX RATIO

From 1,169 adults which emerged in confinement, 48.59 per cent were females and 51.41 per cent males. The occurrence of parthenogenesis may explain the preponderance of males in this species.

To determine whether the several individuals developing in single puparia usually comprised one or both sexes, records were kept of the sex of the adults (whether emerged or not), in the case of 48 host puparia. Of these 48 puparia, 40 yielded adult parasites, each brood of which was either pure male or pure female; while each of the remaining 8 puparia produced a mixed brood of both males and females. This indicates that in the polyembryonic development of *Platygaster vernalis* the adults produced from a single egg are usually of the same sex. The coming of both sexes from a single host could be explained on the grounds that more than one egg was deposited in a single host insect.

#### OVIPOSITION

A female of *Platygaster vernalis*, when seeking Hessian fly eggs in which to oviposit, travels at a moderate rate up and down the leaves of the wheat plant, repeatedly tapping the leaf before her with her antennæ.

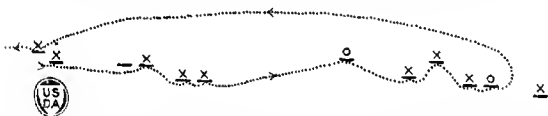


FIG. 1.—Diagram showing the tendency of *Platygaster vernalis* to avoid ovipositing twice into the same egg. The short lines represent the Hessian fly eggs; the crosses mark out the eggs oviposited into on the first visit of the parasite; the dotted line plots out the course of the second visit, the circles mark out the host eggs oviposited into during the second visit.

As soon as her antennæ come in contact with an egg, she halts and concentrates on the spot. At times she loses track of the egg, whereupon she turns in small circles until she finds it again. After finding the egg, the parasite strokes it rapidly with her antennæ and meanwhile strides it with her body held parallel to the long axis of the egg, whereupon the abdomen is drawn up with the ovipositor exerted and in contact with the egg. After sliding the ovipositor back and forth over the surface of the egg several times, she begins to insert it, usually at about the middle of the egg. At this point she draws her antennæ downward and remains motionless for the rest of the time, except for slight movements of her abdomen, while bearing down on her ovipositor. The entire act of oviposition requires about one minute, the average of 10 ovipositions being 59.9 seconds.

Although certain individuals in confinement have deliberately oviposited several times in one host egg, most females seem to avoid ovipositing more than once in the same egg. This tendency is shown by the experiment illustrated in figure 1.

In this diagram 12 Hessian fly eggs are represented in approximately the same position as they occurred on the wheat leaf. A *vernalis* female was allowed to visit these eggs twice. During the first visit the eggs marked with a cross were attacked. The dotted line plots out the course of the second visit, and the circles indicate the eggs attacked during this trip. During the second trip the female examined each of the eggs with the exception of the one at the extreme end, but she consistently refused to oviposit in any except two which had been omitted during the first trip.

Experiments indicate that *Platygaster vernalis* lays only one egg during a single oviposition. This is the usual number found in reared material and in that collected in the field. In confirmation of this belief, it was found that 11 host eggs laid in confinement and dissected immediately after having been punctured by this species contained but one *vernalis* egg each.

#### POTENTIAL PROGENITIVENESS

At the time of eclosion of the adults the eggs have reached their full development in size. In order to ascertain the average number of eggs contained in the ovaries, the eggs of 10 females were dissected out and counted. The count showed an average of 228.3 eggs per female, with a maximum of 290 and a minimum of 117. The eggs are so small and numerous that in order to count them it was necessary to spread them out in a liquid on an eye-piece micrometer disk ruled into 1 millimeter squares. By counting the eggs from one ovary at a time, accurate results were possible.

#### LENGTH OF LIFE

In order to obtain data on the length of life of the adult, 59 adults were divided into three groups subject to different conditions. Lot 1 included 15 females and 4 males, placed in a large dry vial plugged with cotton and with a little sugar solution for nourishment. Lot 2 contained 14 females and 8 males in small vials plugged with cotton and left in a saturated atmosphere, with water accessible. Lot 3 consisted of 15 females and 3 males in small vials in a saturated atmosphere but with sugar solution for nourishment. The atmosphere for Lots 2 and 3 was kept saturated by placing the vials in a relaxing box. The results are summarized in Table II. The shortest length of life was 3 days and the longest 27 days; in Lots 1 and 2 the average length of life of the females exceeded that of the males; and the parasites left in a saturated atmosphere with water available had the longest average length of life, namely, 12.21 days. Throughout the experiment the temperature of the laboratory in which it was conducted varied from 45° F. to 78° F., with an average temperature of 64° F.

Extremely low or high temperatures undoubtedly have some effect on the length of life. Several adults, however, were subjected to a temperature of 24° F. (-4.15° C.) for a period of 5 hours without any noticeable ill effects. Adults subjected to heat expired within a minute at temperatures from 117.68° F. to 120.2° F. (47.6° C. to 49° C.). Short exposures to temperatures of 117.22° F., 116.60° F., and lower did not prove fatal.

TABLE II.—Length of life of *Platygaster vernalis* adults

Lot 1 was kept in a dry atmosphere with sugar solution for nourishment; Lot 2 in a saturated atmosphere with water; and Lot 3, in a saturated atmosphere, with sugar solution for nourishment.]

Lot.	Number of adults.		Length of life in days.				
	Female.	Male.	Maximum.	Minimum.	Average.		
					Female.	Male.	Total.
1	15	4	6	3	4.53	3	4.15
2	14	8	26.33	3	14.71	9.61	12.21
3	15	3	27	3	8.46	8.89	8.53

## OTHER BEHAVIOR

When in confinement the adults crawled rapidly about but very seldom flew. They nearly always moved toward the light. When suddenly disturbed, they feigned death by drawing up the legs and antennae close to the body and remaining in this attitude for a few seconds. In actual death, the antennae and legs are found stretched away from the body. When at rest, the body is usually held in a crouched position, but with legs and antennae not drawn as close to the body as when simulating death.

For nourishment the adults readily take up sugar solution. When allowed to go a few days without water, they became very thirsty, as was demonstrated by the quickness with which they found a drop of water placed near them and the eagerness with which they accepted it.

When emerging, the adult parasite gnaws a round or irregularly shaped exit hole through the cocoon and puparium. (Pl. 3, F.) Sometimes a few such holes in the host puparium are sufficient to permit all the adults within the host to escape.

## SEASONAL HISTORY

To obtain data on the duration of the various stages of *Platygaster vernalis* under normal field conditions, dissections were made of the Hessian fly in various stages collected at intervals throughout the year. Such records were kept during 1918 at Carlisle, Pa., 1919 and 1920 at Mount Holly Springs, Pa.; and 1921 at New Windsor, Md.

The lines on figure 2 show the maximum range of occurrence of each stage as determined by the earliest and latest records of their presence in the field, assembled from data collected during the four years mentioned. The records of the occurrence of adults were obtained by sweeping, and those of the embryos from dissections of Hessian fly larvae after they had descended to the bases of the plants. Since *Platygaster vernalis* oviposits in the egg of its host, embryos of this parasite must have occurred in the field somewhat earlier than is indicated in the figure. It may be observed that the *vernalis* larvae spend considerable time within cocoons before pupating. Cage rearings, checked by field examinations made during the winter, show that the adults normally remain within the cocoons until early spring. The line representing this stage on the chart has not been extended farther than to September 27 for economy



of space. Occasionally adults have been found to emerge and oviposit during the autumn, but this is exceptional, and field observations indicate that eggs deposited at that season fail to mature.

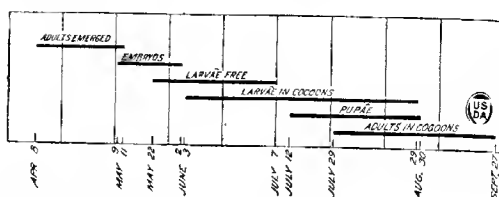


Fig. 2.—Periods of occurrence of the various stages of *Platygaster vernalis* as found from examinations made during the years from 1918 to 1921, inclusive. The dates on which the examinations were made are indicated at the bottom of the diagram.

Figure 3 shows the rate of development of the various stages of *Platygaster vernalis* from May 17 to October 18. These data were obtained by the dissection of Hessian fly stages collected in lots of 100 or more

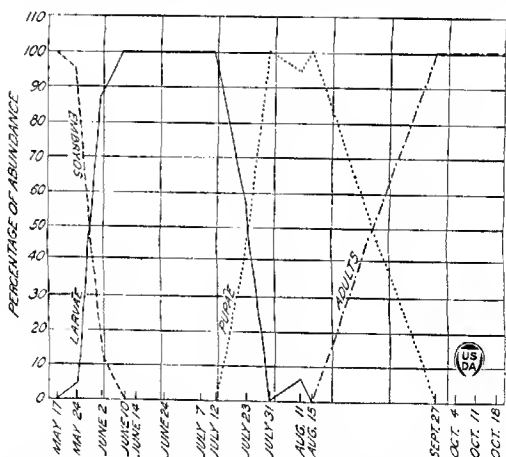


Fig. 3.—Rate of development of the various stages of *Platygaster vernalis* as they occurred during the year 1919 on lawn at Mount Holly Springs, Pa. The dates on which examinations were made are indicated at the bottom of the diagram.

at certain dates during 1919, as indicated at the base of the diagram. All of the collections were made at Mount Holly Springs, Pa. The rather late occurrence of *vernalis* adults during that year was probably due entirely to meteorological conditions.

## CERTAIN ECOLOGICAL CONSIDERATIONS

Various factors in the environment of *Platyaster vernalis* have a marked effect in modifying its normal rate of multiplication and help to produce correspondingly high or low annual rate of mortality.

By making a series of examinations of Hessian fly forms taken from one certain field or farm throughout the season, it has been possible to obtain interesting data bearing on this point. The individual puparia in each examination were selected impartially as they were met with, while the wheat tillers were being inspected for them. They were dissected under the binocular microscope and the contents of each carefully classified. Table III shows the results obtained during the summers of 1918, 1919, 1920, and 1921. The same results are graphically represented in figures 4, 5, 6, and 7. The collections were made on a farm at Carlisle, Pa., in 1918, on a farm at Mount Holly Springs, Pa., in 1919 and 1920, and on a farm at New Windsor, Md., in 1921.

TABLE III.—Mortality of *Platyaster vernalis*, as observed in collections of Hessian fly forms made at intervals during the years 1918, 1919, 1920, and 1921

## 1918: FIVE COLLECTIONS FROM FARM, CARLISLE, PA.

Date of collection.	Fly forms examined.	Results of examination of Hessian fly forms.				
		Containing living <i>P. vernalis</i> .	Containing dead unrecognitionable matter and dead <i>P. vernalis</i> .	Otherwise parasitized.	Living unparasitized fly forms.	Mortality of <i>P. vernalis</i> .
		Number.	Per cent.	Per cent.	Per cent.	Per cent.
May 7	38	79.37	0.00	0.00	20.63	0.00
June 17	184	76.09	10.33	4.89	8.60	4.00
24	52	59.62	15.38	7.69	17.31	24.83
July 19	18	44.44	0.00	50.00	5.56	43.90
Aug. 27	81	14.81	28.40	53.09	3.70	84.33

## 1919: NINE COLLECTIONS FROM FARM, MOUNT HOLLY SPRINGS, PA.

May 27	139	57.55	1.44	0.00	41.01	0.00
June 5	100	46.00	4.00	4.00	46.00	20.07
12	110	28.18	15.46	8.18	48.18	51.03
July 11	100	23.00	21.00	36.00	20.00	60.03
21	100	22.00	23.00	46.00	9.00	61.77
Aug. 7	150	12.67	27.33	52.67	7.33	77.98
11	200	11.00	25.00	60.00	4.00	80.89
Sept. 23	200	4.00	29.50	62.50	4.00	93.05
Oct. 10	200	3.50	27.50	66.50	2.50	93.91

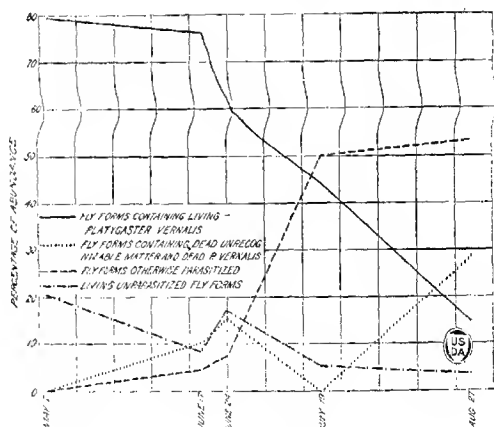
## 1920: FIVE COLLECTIONS FROM FARM, MOUNT HOLLY SPRINGS, PA.

June 10	14	51.00	6.00	7.00	36.00	0.00
July 21	186	7.51	40.86	50.55	1.08	85.27
Aug. 31	200	9.00	51.00	38.00	2.00	82.35
Sept. 8	100	4.00	51.00	45.00	.00	92.16
Nov. 27	100	2.00	55.00	42.00	.00	96.08

TABLE UI.—Mortality of *Platygaster vernalis*, as observed in collections of Hessian fly forms made at intervals during the years 1918, 1919, 1920, and 1921—Continued

1921: EIGHT COLLECTIONS FROM FARM, NEW WINDSOR, MD.—Continued.

Date of collection.	Results of examination of Hessian fly forms.					
	Fly forms examined.	Containing living <i>P. vernalis</i> .	Containing dead unrecognizable matter and dead <i>P. vernalis</i> .	Otherwise parasitized.	Living unparasitized fly forms.	Mortality of <i>P. vernalis</i> .
	Number.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
May 9.....	117	76.07	15.39	6.85	7.69	0.00
June 3.....	100	65.00	21.00	16.00	8.00	17.00
21.....	100	29.00	25.00	40.00	8.00	61.00
July 12.....	100	16.00	43.00	38.00	3.00	78.00
28.....	100	16.00	36.00	26.00	2.00	78.00
Aug 12.....	110	20.91	46.36	28.18	4.55	72.73
30.....	100	21.00	47.00	29.00	3.00	72.00
Sept. 23.....	100	6.00	70.00	21.00	3.00	93.00

FIG. 4.—Mortality of *Platygaster vernalis*, as observed in collections of Hessian fly forms made at Carlisle Pa., in 1918. (Table III.)

In all cases, for the sake of the uniformity necessary in obtaining correct percentage results, the host is taken as the unit. For instance if all the individual parasites in a single host were dead, the latter was classified as containing dead *vernalis*, but if one or more of the *vernalis* were alive, the host was classified as containing the living parasite.

Under the heading "Otherwise parasitized" are included puparia from which parasites other than *vernalis* have emerged, or in which such other parasites are recognized whether dead or alive. In case of the recognizable occurrence of both *vernalis* and some other parasite in the

same host, it was classified according to the condition of *vernalis*. Such cases were comparatively few.

The number of hosts containing dead material recognized as *vernalis* was, as a rule, exceedingly small. The cause of the death of *vernalis* in such cases could seldom be determined, although sometimes they appeared to have been eaten by predators.

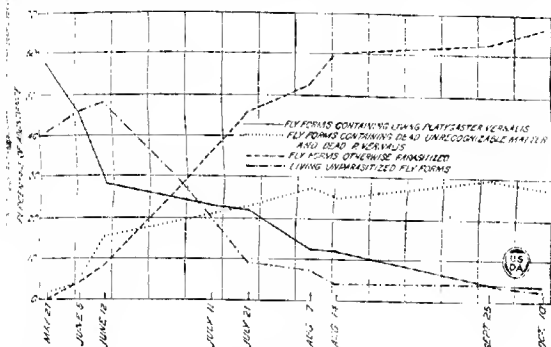


FIG. 1.—Mortality of *Platygaster vernalis* as observed in collections of Hessian fly forms made at Mount Holly Springs, Pa., in 1919. (Table III.)

In 1918 (Table III), by the 19th of July, the mortality of *Platygaster vernalis* reached 43.96 per cent and by August 27, the date of the last collection for that year, it had reached as high as 81.33 per cent. At that time the death of at least 45.51 per cent of the *vernalis* parasites was due to the competition of other Hessian fly parasites. It would be hazardous to say that any greater proportion were destroyed by this agency,

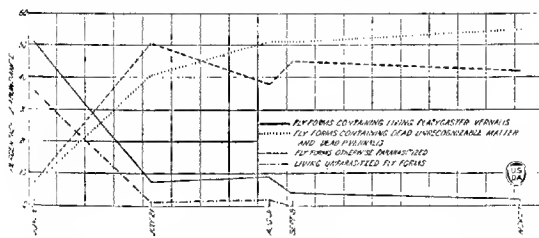


FIG. 2.—Mortality of *Platygaster vernalis*, as observed in collection of Hessian fly forms made at Mount Holly Springs, Pa., in 1920. (Table III.)

since the remaining 35.82 per cent could be accounted for by hosts containing dead unrecognizable matter and dead *vernalis*.

In 1919 (Table III), by July 11, 60.03 per cent of the *vernalis* had been destroyed. By October 10 of the same year 93.91 per cent had died. Of these at least 46.14 per cent were killed in competition with other hymenopterous parasites of the Hessian fly.

In 1920 (Table III), by July 21, 85.27 per cent were destroyed, and by the 27th of November the mortality had reached 96.08 per cent. Although other parasites undoubtedly were responsible for the death of many of these, the figures on this date show such a high percentage of puparia containing dead, unrecognizable matter and dead *Platygaster vernalis* that the entire 96.08 per cent could not be assumed as having been killed in competition with other parasites.

In 1921 by the 12th of July (Table III), there was a mortality of *Platygaster vernalis* to the extent of 78.97 per cent and by September 23 the percentage of death reached 92.11 per cent. While in the results of this last examination the death of nearly all of the *vernalis* could be accounted for by the large number of puparia containing dead unrecognizable matter and dead *P. vernalis*, yet the figures from the collection taken June 21 of the same year show that fully 31.64 per cent had already been killed as early as June by other hymenopterous parasites.

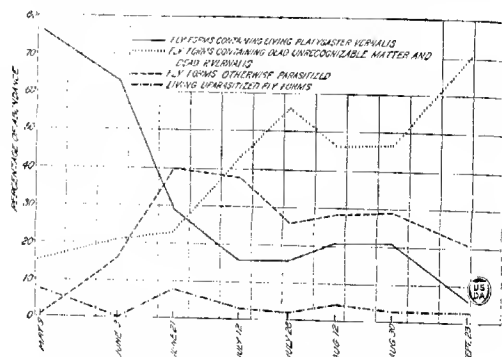


FIG. 7.—Mortality of *Platygaster vernalis* as observed in collections of Hessian fly forms made at New Windsor, Md., in 1920. (Table III.)

#### SUMMARY

From the foregoing observations the following conclusions may be deduced:

- (1) During each year the death rate of *Platygaster vernalis* was very high, being not less than 81.33 per cent for any one year, and in 1920 being as high as 96.08 per cent.
- (2) A large percentage of the mortality of *P. vernalis* was due to competition with other Hessian fly parasites.
- (3) During the years 1918, 1920, and 1921 for the localities under observation, *P. vernalis* was more effective than all other parasites of the spring generation of the Hessian fly combined.

It should be stated that although the attacks of the other parasites are highly detrimental to the multiplication of *Platygaster vernalis*, yet they supplement the latter sufficiently well to effect a very high death rate of the Hessian fly. They also act as a safeguard in case of scarcity of *vernalis*. In all cases, moreover, the hyperparasitism appears to be entirely accidental. It would therefore be unwise to discount too greatly the value of the other parasites.



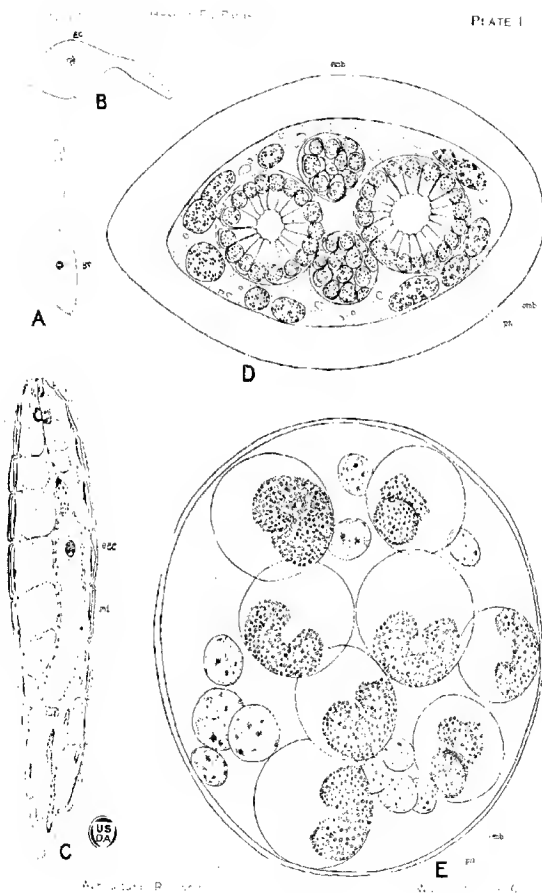
# PLATE 1

## *Platyaster vernalis*

- A. Longitudinal section of egg before oviposition.  $\times 397$ .  
 B. Longitudinal section of egg immediately after oviposition.  $\times 391$ .  
 C. Longitudinal section of Hessian fly larva containing a *vernalis* egg within the midintestine.  $\times 29$ .  
 D. Sagittal section of *Platyaster vernalis* egg about 11 days old, showing four embryos in the blastula stage of development and numerous paranuclear masses present.  $\times 695$ .  
 E. Paranuclear masses of *Platyaster vernalis* containing eight embryos at an advanced stage of development.  $\times 87$ .

## Explanation of symbols on Plates 1-4

egg=egg.	mx=maxilla.
emb=embryo.	pn=paranuclear mass.
gc=germ cell.	pr=proctodaeum.
go=germ cell.	sh=sheath.
mb=maxillule.	sq=sternal spatula.
mi=midintestine.	stw=stomach wall.
mth=mouth.	styl=stylet.





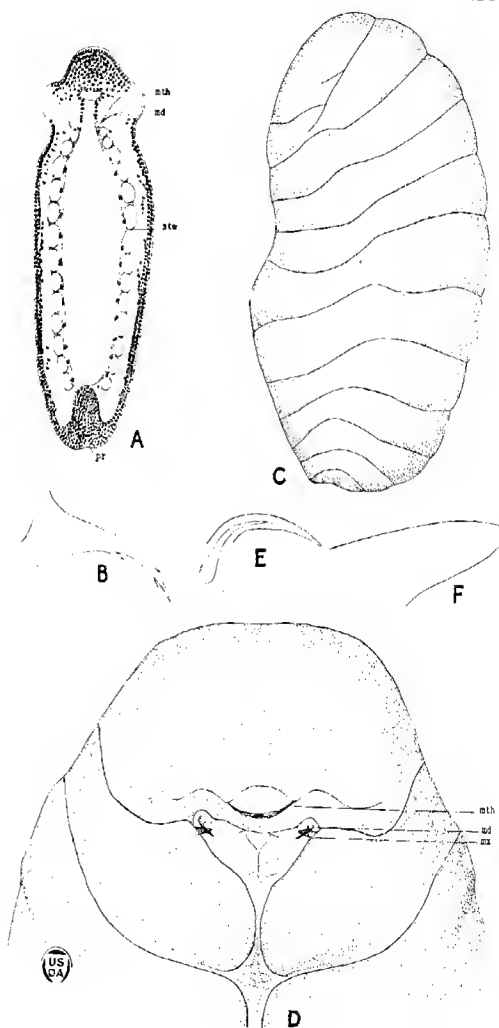


PLATE 2

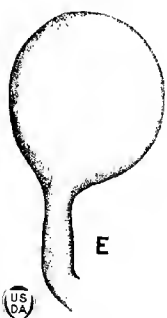
*Platygaster vernalis*:

- A.—Ventral view of primary larva, showing certain outstanding features of the internal anatomy, such as the stomach wall and proctodacum.  $\times 132$ .  
B.—Mandible of primary larva.  $\times 417$ .  
C.—Lateral aspect of mature larva.  $\times 77$ .  
D.—Ventral aspect of head and thorax of mature larva, showing mouth and mouth parts.  $\times 242$ .  
E.—Mandible of mature larva.  $\times 833$ .  
F.—Maxilla of mature larva.  $\times 833$ .

PLATE 3

*Platypaster vernalis*:

- A. *Platypaster vernalis* cocoons from a single Hessian fly puparium.  $\times 20$ .
- B. Lateral aspect of *P. vernalis* pupa.  $\times 96$ .
- C. Ovipositor of *P. vernalis*.  $\times 317$ .
- D.—Parts of ovipositor of *P. vernalis*, showing sheath, gorgeret, and stylet.
- E.—Ovary of *P. vernalis*.  $\times 346$ .
- F.—Exit holes in Hessian fly puparium made by adult *P. vernalis* in emerging.  $\times 13$ .



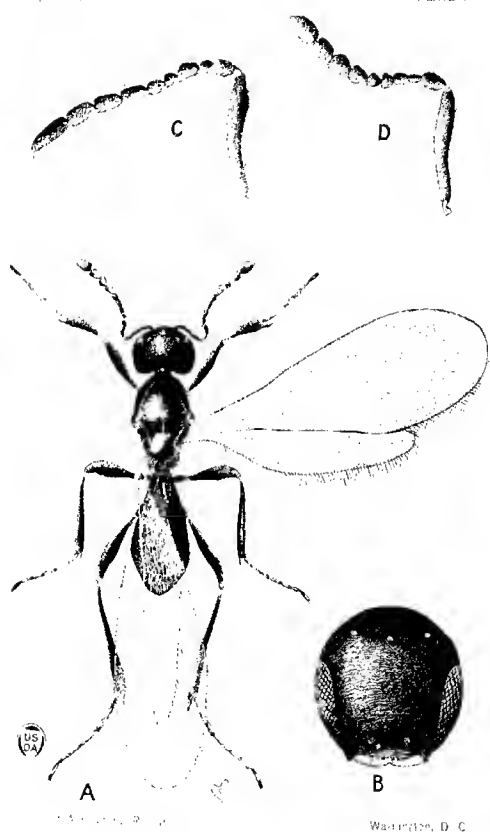


PLATE 4

*Platygaster zentralis*:

- A.—Adult female.
- B.—Frontal view of head of adult.
- C.—Antenna of male.
- D.—Antenna of female.

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